REMARKS

This Amendment responds to the Office Action mailed March 14, 2008 (the Office Action). The Office Action was carefully reviewed: Applicant has attempted to expedite prosecution and meet every concern that the Patent Office has set forward.

Status of the Claims

Claims 17, 19, 21-24, 29-38, and 65 are under examination. Claim 65 has been allowed. New claims 66-73 are added to itemize previously claimed components of claim 21. Claim 21 is amended to move certain components to new dependent claim 66.

Overview

An interview, of record, indicated that the Patent Office might find more information to be helpful with respect to examination of the enablement issues. Two sets of experiments are provided that support the enablement and use of different materials in the claimed invention. Further publications are provided that provide further detail about additional fillers and how they are suited to what is claimed. One of these publications shows that hyaluronic acid (see claim 32) was successfully used in a location between the rectum and prostate. This information is provided by way of Dr. Sawhney's Declaration, attached, and a review of the instant Application as well as the legal standards that the Patent Office must apply.

Rejection for lack of enablement

Claims 17-25, 27, 29-38, and 62-64 were rejected under 35 U.S.C. §112 ¶1 on the grounds of nonenablement for materials other than collagen. The Office Action argues that

Applicant has only established ample support for collagen, as at pages 5-6, with support for other embodiments being generally lacking. Coverage of "polyethylene glycol" in claim 21 was specifically rejected (see also new claim 71).

The entire rationale set forth in the Office Action is: "The specification merely states at page 6, lines 7-9, that 'Other materials may be used that include natural or synthetic materials, e.g., proteins. . . polyethylene glycol-based materials'. This disclosure is not sufficient to support the species of filler materials of instant claim 21 and is found unpatentable." The Declaration includes objective third-party evidence that hyaluronic acid can be used as claimed, see *I. J. Radiation Oncology*, attached, and as described at item 4b of the Declaration. This published report demonstrates that hyaluronic acid (see claim 32) was successfully used in a location between the rectum and prostate. This evidence, by itself, is evidence that the disclosure of more than about 20 fillers detailed below is enabling for the genus of independent claim 17. Other objective evidence is described below.

Legal Standard of Enablement

MPEP 2164.01 describes the test for enablement as follows. Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention. The standard for determining whether the specification meets the enablement requirement was cast in the Supreme Court decision of Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916) which postured the question: is the experimentation needed to practice the invention undue or unreasonable. MPEP 2164.01(a) describes the Wands factors for undue experimentation.

MPEP 2164.04 describes the Patent Office's burden as follows. In order to make a rejection, the examiner has the initial burden to establish a reasonable basis to question the

enablement provided for the claimed invention. In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993) (Examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

All Evidence Must Be Considered

MPEP 716.01(B) explains that evidence must be considered in detail:

> (B) *Consideration of evidence.* **Evidence traversing rejections**, when timely presented, **must be considered by the examiner whenever present**. All entered affidavits, declarations, and **other evidence traversing rejections** are acknowledged and commented upon by the examiner in the next succeeding action. The extent of the commentary depends on the action taken by the examiner. Where an examiner holds that the evidence is sufficient to overcome the *prima facie* case, the comments should be consistent with the guidelines for statements of reasons for allowance. See MPEP § 1302.14. Where the evidence is insufficient to overcome the rejection, **the examiner must specifically explain why the evidence is insufficient. General statements** such as "the declaration lacks technical validity" or "the evidence is not commensurate with the scope of the claims" without an explanation supporting such findings **are insufficient**. MPEP 716.01(B), emphases added.

I. THE PATENT OFFICE'S RATIONALE FOR THE ENABLEMENT REJECTION IS CLEARLY ERRONEOUS.

Respectfully, the Office Action points to support in the specification but provides no explanation as to why this support is not enabling. Such an approach falls far short of even a minimal basis for the Patent Office to make-out a valid rejection. The Patent Office is obligated to allow the claims unless it has good reasons for believing them not to be enabled. The Patent

Office's argument that "This disclosure is not sufficient to support the species of filler materials of instant claim 21 and is found unpatentable" is a conclusion and not a reason. This problem with the rationale is, by itself, a basis for allowing the claims.

II. THE ATTACHED DECLARATION PROVIDES DETAILED EXPLANATION AND OBJECTIVE EVIDENCE IN THE FORM OF PUBLICATIONS THAT DEMONSTRATE ENABLEMENT; THIS OBJECTIVE EVIDENCE MUST BE CONSIDERED AND RESPONDED TO IN DETAIL.

The Sawhney Declaration explains that many fillers are described in the Application that can be used besides collagen, as is evident from the fact that collagen was actually tested and many other fillers also have the relevant properties of being biocompatible, biodegradable, and introducible in a flowable state (for example, via a syringe needle or catheter) to form a material that can displace tissues relative to each other and also stay in the intended location. The Sawhney Declaration further discusses collagen, see Declaration item 4a and also cites to Sclafani et al., with reference to collagen's role as a filler.

The Sawhney Declaration see Declaration, item 4b, also points to Prada et al., which describes a detailed peer-reviewed set of experiments that document the success of hyaluronic acid used as a filler in a space between the rectum and prostate (see claims 31-32). Thus both collagen and hyaluronic acid have been specifically documented by experimental data that underpins the Applicant's claims and points to broad enablement of fillers, many of which are available to artisans. These facts, by themselves, clearly support the claimed genus of fillers.

The Declaration nonetheless provides yet more evidence and describes how polysaccharides can be used and points out that hyaluronic acid and alginates are polysaccharides. Declaration, item 4c The specification provides detailed guidance for use of polysaccharides, as explained below. The Declaration also speaks to fibrin or fibrinogen (claims

21, 69), Declaration item 4d, referencing also Canonico and U.S. Pat. No. 4,874,368. The Declaration also speaks to albumin or gelatin (claims 21, 68, 70), Declaration, item 4e, referencing also Funovics et al. and U.S. Pat. No. 5,618,551.

The Declaration also speaks to various polyethylene glycol materials (claims 21, 31, 71), Declaration, item 4f, referencing also Kacher et al. and U.S. Pat. No. 5,410,016 for which Sawhney is an inventor. Detailed experimental results that describe polyethylene glycol materials used as fillers and having properties suited to the claimed invention are provided and discussed Declaration, item 4f, see "Polyethylene Glycol Materials" report. As explained in detail in the Declaration, the experiments show that the polyethylene glycol materials have physical properties that are comparable to collagen or hyaluronic acid for purposes of the claimed invention.

The Declaration also speaks to thixotropic polymers or thermoreversible polymers (claims 21, 72-73), Declaration item 4g. Such materials include hyaluronic acid, which is described in Prada et al., which is claimed, and which is described in the Application as detailed below. Such materials include PLURONICS or TETRONICS materials that are described in detail in the Application as explained below.

III. THE APPLICATION PROVIDES DETAILED ENABLEMENT FOR FILLERS AS CLAIMED.

The specification does support and enable what is claimed. This evidence, by itself, is a basis for allowing the claims.

As explained on page 6 of the Application (lines 6-10), "The successful use of collagen as a filler shows that other materials may also be used. Other materials may include natural or synthetic materials, e.g., proteins, extracellular matrix molecules, fibrin, proteins, hyaluronic acid, albumin, bulking agents, and polyethylene glycol-based materials". And "A filler is a substance that occupies a volume after its introduction into a body. Examples of fillers include

but are not limited to polymers, gels, sols, hydrogels, sponges, bulking agents, and balloons. Filler materials include polysaccharides, alginate, collagen, gelatin, fibrin, fibrinogen, albumin, serum, autologous serum, sutures, and natural and synthetic polymers. Synthetic polymers include polylactide, polyglycolide, polycaprolactones, poly(alpha.-hydroxy acid), poly(amino acid), and poly(anhydride). Fillers may be crosslinked or uncrosslinked. Polymers include polyethylene glycol and derivatives thereof, including crosslinked polyethylene glycols. Other types of polymers include thermoreversible and thixotropic polymers. Other examples of a filler include self-absorbing suture material held within a suspension (such as prolene sutures)", see specification page 7 line 20-page 8 line 7.

Fillers from commercial sources are disclosed in the specification at pages 11-21: "Other examples of fillers are hyaluronic acid, cellulose, alginate, and gelatin, which are available from commercial sources, e.g., Sigma-Aldrich, Inc. and ICN Biomedicals, Inc. Hyaluronic acid is a material that is accepted in the medical community as a material that may be implanted into a patient; other commercial sources are Genzyme Advanced Biomaterials (e.g., HyluMed®), LifeCore Biomedical, and FMC BioPolymer. Another example of a filler is cellulose, e.g., Avicel® a thixotropic cellulose product from FMC BioPolymer. Another filler example is synthetic polymer hydrogels, e.g., as made by Angiotech Pharmaceuticals, e.g., Coseal®. Other fillers are described in, e.g., U.S. Patent No. 6,224,893, and other references set forth herein.

The Office action pointed particularly to "polyethylene glycol" as not being enabled. The passage just quoted refers to Coseal®, a polyethylene glycol-material (see attached Coseal® literature, 2 documents). Further, material incorporated by reference and copied into the specification describes "For example, a mixture of polyethylene oxide and polyacrylic acid which gels by hydrogen bonding upon mixing may be utilized" and "PEG-oligolactyl-acrylates", "polyalkylene oxalates", and "polyalkylene succinates" are described, with a polyethylene glycol being a well-known type of polyalkylene.

Additional detailed support enabling the artisan to practice what is claimed has been included into the Application. The artisan may follow the working examples or the specification to make a degradable device as claimed using various fillers. The fillers that are disclosed include **more than about 20 specific embodiments that may be obtained using methods detailed in the literature or merely by ordering products that are commercially available.** Therefore it is not undue experimentation to make and practice what is claimed.

Referring to the Application, as included at page 11, line 12 (emphases added):

Polymeric materials which are capable of forming a hydrogel may be utilized. In one embodiment, the polymer forms a hydrogel within the body upon contact with a crosslinking agent. A hydrogel is defined as a substance formed when an organic polymer (natural or synthetic) is crosslinked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. **Naturally occurring and synthetic hydrogel forming polymers, polymer mixtures and copolymers may be utilized as hydrogel precursors**.

Examples of materials which can be used to form a hydrogel include modified alginates. Alginate is a carbohydrate polymer isolated from seaweed, which can be crosslinked to form a hydrogel by exposure to a divalent cation such as calcium, as described, for example in WO 94/25080. Alginate is ionically crosslinked in the presence of divalent cations, in water, at room temperature, to form a hydrogel matrix. Modified alginate derivatives may be synthesized which have an improved ability to form hydrogels. The use of alginate as the starting material is advantageous because it is available from more than one source, and is available in good purity and characterization. As used herein, the term "modified alginates" refers to chemically modified alginates with modified hydrogel properties. Naturally occurring alginate may be chemical modified to produce alginate polymer derivatives that degrade more quickly. For

example, alginate may be chemically cleaved to produce smaller blocks of gellable oligosaccharide blocks and a linear copolymer may be formed with another preselected moiety, e.g. lactic acid or .epsilon.-caprolactone. The resulting polymer includes alginate blocks which permit ionically catalyzed gelling, and oligoester blocks which produce more rapid degradation depending on the synthetic design. Alternatively, alginate polymers may be used, wherein the ratio of mannuronic acid to guluronic acid does not produce a firm gel, which are derivatized with hydrophobic, water-labile chains, e.g., oligomers of .epsilon.-caprolactone. The hydrophobic interactions induce gelation, until they degrade in the body.

Additionally, polysaccharides which gel by exposure to monovalent cations, including bacterial polysaccharides, such as gellan gum, and plant polysaccharides, such as carrageenans, may be crosslinked to form a hydrogel using methods analogous to those available for the crosslinking of alginates described above. Polysaccharides which gel in the presence of monovalent cations form hydrogels upon exposure, for example, to a solution comprising physiological levels of sodium. Hydrogel precursor solutions also may be osmotically adjusted with a nonion, such as mannitol, and then injected to form a gel.

Polysaccharides that are very viscous liquids or are **thixotropic,** and form a gel over time by the slow evolution of structure, **are also useful**. For example, **hyaluronic acid,** which forms an injectable gel with a consistency like a hair gel, may be utilized. Modified hyaluronic acid derivatives are particularly useful. As used herein, the term "modified hyaluronic acids" refers to chemically modified hyaluronic acids. **Modified hyaluronic acids may be designed and synthesized with preselected chemical modifications to adjust the rate and degree of crosslinking and biodegradation.** For example, modified hyaluronic acids may be designed and synthesized which are esterified with a relatively hydrophobic group such as propionic acid or benzylic acid to render the polymer more hydrophobic and gel-forming, or which are grafted with amines to promote electrostatic self-assembly. Modified hyaluronic acids thus may be

synthesized which are injectable, in that they flow under stress, but maintain a gel-like structure when not under stress. Hyaluronic acid and hyaluronic derivatives are available from Genzyme, Cambridge, Mass. and Fidia, Italy.

Other polymeric hydrogel precursors include polyethylene oxide-polypropylene glycol block copolymers such as PLURONICS or TETRONICS, which are crosslinked by hydrogen bonding and/or by a temperature change, as described in Steinleitner et al., Obstetrics & Gynecology, 77:48-52 (1991); and Steinleitner et al., Fertility and Sterility, 57:305-308 (1992).

Other materials which may be utilized include proteins such as fibrin, collagen and gelatin. Polymer mixtures also may be utilized. For example, a mixture of polyethylene oxide and polyacrylic acid which gels by hydrogen bonding upon mixing may be utilized. In one embodiment, a mixture of a 5% w/w solution of polyacrylic acid with a 5% w/w polyethylene oxide (**polyethylene glycol,** polyoxyethylene) 100,000 can be combined to form a gel over the course of time, e.g., as quickly as within a few seconds.

Covalently crosslinkable hydrogel precursors also are useful. For example, a water soluble polyamine, such as chitosan, can be cross-linked with a water soluble diisothiocyanate, such as **polyethylene glycol** diisothiocyanate. The isothiocyanates will react with the amines to form a chemically crosslinked gel. Aldehyde reactions with amines, e.g., with **polyethylene glycol** dialdehyde also may be utilized. A hydroxylated water soluble polymer also may be utilized.

Alternatively, polymers may be utilized which include substituents which are crosslinked by a radical reaction upon contact with a radical initiator. For example, polymers including ethylenically unsaturated groups which can be photochemically crosslinked may be utilized, as disclosed in WO 93/17669. In this embodiment, water soluble macromers that include at least one water soluble region, a biodegradable region, and at least two free radical-polymerizable

regions, are provided. The macromers are polymerized by exposure of the polymerizable regions to free radicals generated, for example, by photosensitive chemicals and or light. Examples of these macromers are **PEG-oligolactyl-acrylates,** wherein the acrylate groups are polymerized using radical initiating systems, such as an eosin dye, or by brief exposure to ultraviolet or visible light. Additionally, water soluble polymers which include cinnamoyl groups which may be photochemically crosslinked may be utilized, as disclosed in Matsuda et al., ASAID Trans., 38:154-157 (1992).

Water soluble polymers with charged side groups may be crosslinked by reacting the polymer with an aqueous solution containing ions of the opposite charge, either cations if the polymer has acidic side groups or anions if the polymer has basic side groups. Examples of cations for crosslinking of the polymers with acidic side groups to form a hydrogel are monovalent cations such as sodium, and multivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, and di-, tri- or tetra-functional organic cations such as alkylammonium salts. Aqueous solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Additionally, the polymers may be crosslinked enzymatically, e.g., fibrin with thrombin.

Referring to the Application, as included after page 10, line 12, emphases added:

Thermoplastic polymers include pharmaceutically compatible polymers that are bioerodible by cellular action, are biodegradable by action of non-living body fluid components, soften when exposed to heat but return to the original state when cooled and are capable of substantially dissolving or dispersing in a water-miscible carrier or solvent to form a solution or dispersion. Upon contact with an aqueous fluid and the dissipation of the solvent component, the thermoplastic polymers are capable of coagulating or solidifying to form a solid or gelatinous

matrix suitable for use as an implant in an animal. The kinds of thermoplastic polymers suitable for the present composition generally include any having the foregoing characteristics. Examples are **polylactides, polyglycolides, polycaprolactones, polyanhydrides,** polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, **polyalkylene oxalates, polyalkylene succinates,** poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures therein. **Polylactides, polycaprolactones, polyglycolides** and copolymers thereof are highly preferred thermoplastic polymers.

The thermoplastic polymer is combined with a suitable organic solvent to form a solution. The solubility or miscibility of a polymer in a particular solvent will vary according to factors such as crystallinity, hydrophilicity, capacity for hydrogen-bonding and molecular weight of the polymer. Consequently, the molecular weight and the concentration of the polymer in the solvent are adjusted to achieve desired miscibility. Highly preferred thermoplastic polymers are those which have a low degree of crystallization, a low degree of hydrogen-bonding, low solubility in water, and high solubility in organic solvents.

The composition of the invention may as well be a liquid formulation of a thermosetting oligomeric pre-polymer or copolymer which is capable of cross-linking or hardening to provide a microporous gelatinous or solid matrix suitable for use as an implant in an animal, including a human. The thermosetting pre-polymers and resulting cross-linked polymers and copolymers are biocompatible, and biodegradable and/or bioerodible.

The pre-polymers are preferably low molecular weight polymers or oligomers having end functional groups that are reactive with acryloyl chloride to produce acrylic ester-terminated pre-polymers. Acrylic pre-polymers for use in the compositions may be synthesized according to a

variety of methods including, but not limited to, reaction of a carboxylic acid, such as acrylic or methacrylic acid, with an alcohol; reaction of a carboxylic acid ester, such as methyl acrylate or methyl methacrylate, with an alcohol by transesterification; and reaction of an isocyanatoalkyl acrylate, such as isocyanatoethyl methacrylate, with an alcohol.

The thermosetting prepolymers are also short chain polyol derivatives of the thermoplastic polymers described herein. The polyol terminated derivatives are converted to acrylic ester terminated prepolymers by any suitable method. Examples are short chain polyol derivatives of **polylactides, polyglycolides, polycaprolactones, polyanhydrides,** polyamides, polyurethanes, polyesteramides, polyorthoesters, polydioxanones, polyacetals, polyketals, polycarbonates, polyorthocarbonates, polyphosphazenes, polyhydroxybutyrates, polyhydroxyvalerates, polyalkylene oxalates, polyalkylene succinates, poly(malic acid), poly(amino acids), poly(methyl vinyl ether), poly(maleic anhydride), chitin, chitosan, and copolymers, terpolymers, or combinations or mixtures therein.

A polymer matrix and implant prepared with thermosetting prepolymers is composed of poly(DL-lactide-co-caprolactone) (DL-PLC). To prepare the DL-PLC polymer matrix, DL-lactide or L-lactide and .gamma.-caprolactone are co-polymerized in the presence of a multifunctional polyol initiator and a curing agent to produce hydroxy-terminated PLC prepolymers. This polyol-terminated pre-polymer is then converted to an acrylic ester-terminated pre-polymer by any suitable method, as for example, by acylation of the alcohol terminus with acryloyl chloride by means of, for example, a Schotten-Baumann technique (reaction of acyl halide with alcohol).

Optionally, a curing agent, such as a catalyst, may be added to the acrylic pre-polymer mixture to enhance cross-linking of the pre-polymers and the subsequent coagulation or solidification of the resulting polymer to form a matrix. For example, the acrylic pre-polymer, in an amount of about 5 grams, may be added to a solution of benzoyl peroxide (BP) in about 1 ml

of CH_2Cl_2 Optionally, other acrylic monomers may be added to the acrylic pre-polymer mixture before adding the curing agent. The acrylic pre-polymer mixture may be cured in air at room temperature, or in a preheated vacuum oven.

Preferred catalysts for the preparation of the PLC prepolymers are basic or neutral ester-interchange (transesterification) catalysts, as for example, metallic esters of carboxylic acids containing up to 18 carbon atoms, formic, acetic, lauric, stearic, and benzoic acid. Preferred catalysts include, for example, stannous octoate and stannous chloride.

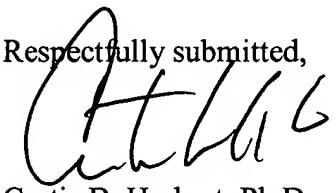
A multi-functional polyol chain initiator may be included in the thermosetting polymer compositions to vary the molecular weight and composition of the polymer. For example, a bifunctional chain initiator such as ethylene glycol, may be included to produce a bifunctional polymer, or a trifunctional initiator, such as trimethylolpropane, may be used to produce a trifunctional polymer. Further, the molecular weight of the polymer or co-polymer may be varied according to the concentration of the chain initiator in the composition. For example, a high concentration of a bifunctional chain initiator may make available an initiator molecule for each polymer chain, while a low concentration may contain one initiator molecule for every two polymer chains.

Following the addition of the curing agent, the pre-polymer polymer mixture preferably remains in liquid form for a period of time effective to allow administration of the composition to the implant site. Thereafter, the cross-linking reaction preferably continues until a solid or gelatinous polymer matrix is produced. Accordingly, the pre-polymer mixture cures, or solidifies, in situ to form a polymer matrix which is capable of biodegradation and/or bioabsorption over time.

Request for allowance

Allowance of the claims is requested. The Examiner is invited to telephone the undersigned if the Examiner believes it would be useful to advance prosecution.

Respectfully submitted,



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CLINICAL INVESTIGATION **Prostate**

TRANSPERINEAL INJECTION OF HYALURONIC ACID IN ANTERIOR PERIRECTAL FAT TO DECREASE RECTAL TOXICITY FROM RADIATION DELIVERED WITH INTENSITY MODULATED BRACHYTHERAPY OR EBRT FOR PROSTATE CANCER PATIENTS

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Purpose: Rectal toxicity remains a serious complication affecting quality of life for prostate cancer patients treated with radiotherapy. We began an investigational trial injecting hyaluronic acid (HA) in the perirectal fat to increase the distance between the prostate and the anterior rectal wall. This is the first report using HA injection in oncology.

Methods and Materials: This is a trial of external beam radiation therapy with HDR brachytherapy boosts in prostate cancer. During the two high-dose-rate (HDR) fractions, thermoluminescent dosimeter dosimeters were placed in the urethra and in the rectum. Before the second HDR fraction, 3–7 mL (mean, 6 mL) of HA was injected under transrectal ultrasound guidance in the perirectal fat to systematically create a 1.5-cm space. Urethral and rectal HDR doses were calculated and measured. Computed tomography and magnetic resonance imaging were used to assess the stability of the new space.

Results: Twenty-seven patients enrolled in the study. No toxicity was produced from the HA or the injection. In follow-up computed tomography and magnetic resonance imaging, the HA injection did not migrate or change in mass/shape for close to 1 year. The mean distance between rectum and prostate was 2.0 cm along the entire length of the prostate. The median measured rectal dose, when normalized to the median urethral dose, demonstrated a decrease in dose from 47.1% to 39.2% ($p < 0.001$) with or without injection. For an HDR boost dose of 1150 cGy, the rectum mean Dmax reduction was from 708 cGy to 507 cGy, $p < 0.001$, and the rectum mean Dmean drop was from 608 to 442 cGy, $p < 0.001$ post-HA injection.

Conclusion: The new 2-cm distance derived from the HA injection significantly decreased rectal dose in HDR brachytherapy. Because of the several-month duration of stability, the same distance was maintained during the course of external beam radiation therapy. © 2007 Elsevier Inc.

Prostate cancer, Brachytherapy, External radiation, Rectal protection and toxicity, Hyaluronic acid.

INTRODUCTION

During the last decade, high-dose-rate (HDR) and low-dose-rate (LDR) prostate brachytherapy have appreciated worldwide renaissance as prostate cancer treatments. This has been documented in the urology (1) and radiotherapy literature (2). It has been used as monotherapy for favorably staged patients (3–5) or as a boost to external beam radiation therapy (EBRT) for intermediate- and high-risk patients (6–12). They are considered within the standard of care for prostate cancer patients (13–19).

Although prostatectomy in any of its forms remains a highly accepted therapy, the shorter recovery time from brachytherapy and the significant decrease in toxicity from brachytherapy has made this treatment more acceptable to patients. In particular, the extremely low rates of urinary incontinence and decreased frequency of erectile dysfunction make brachytherapy a more appealing treatment for prostate cancer patients (5, 13, 20).

The main complications of radiotherapy are related to genitourinary dysfunction or rectal damage. Although techniques to decrease toxicity (*e.g.*, peripheral loading, intraoperative real time dosimetry, and others) have been described, they are predominantly with the intention to decrease urethral damage (21–23). However, attempts to decrease rectal toxicity have been less successful (24, 25).

The majority of publications are in agreement that, re-

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gardless of the brachytherapy technique used (HDR or LDR as monotherapy or in combination with EBRT), rectal complications leading to rectal ulcers are low; perhaps below 2%. However, proctitis Grade 1, 2, or 3 by the Radiation Therapy Oncology Group or by the common toxicity criteria (version 2) are not insignificant, and seen between 2% and 17%. Soiling, mucus loss, and rectal incontinence are also seen. In addition, rectal bleeding reported in up to 20%, although not always of considerable clinical significance, is quite often a further source of distress for the patient and family members, with an important negative impact in quality of life (26–34).

The aforementioned toxicity and disruption of patients' quality of life were the impetus for undertaking the novel approach described in this article. This is the first report in the medical literature using hyaluronic acid (HA) injection in oncology treatments. By injecting HA in the perirectal fat before any type of radiation treatment, thus increasing the distance from the rectal wall to either the radioactive sources or the external beam, we believe much of the side effects can be either reduced or even eliminated. An added advantage of this approach is that the substance remains in the perirectal space without change in shape or absorption for up to 10–12 months, allowing a consistent and systematic displacement during the biologic life of the isotope or course of external beam. Because of the stability of the compound, it is not subject to daily variations in volume or shape and provides a consistent daily distance between the posterior capsule of the prostate and the anterior rectal wall.

METHODS AND MATERIALS

We are describing a new approach to decrease rectal toxicity and improve quality of life for patients with prostate cancer treated with any form of radiotherapy. Transperineal HA injection into the perirectal fat was used to consistently displace the rectal wall away from the effective and biologic life of radiation sources or the length of external beam course. In low and low-intermediate risk patients treated with monotherapy (HDR or LDR), the HA injection was administered either before the implantation of LDR seeds, or before dose delivery for HDR patients. For patients with intermediate- or high-risk disease, the injection was administered during the second intensity-modulated HDR brachytherapy boost treatment as part of an institutional review board–approved study (Table 1).

Study model

External beam interdigitated with intensity-modulated HDR boost: For several years, patients diagnosed with intermediate- and high-risk prostate cancer have been treated at our institution with EBRT interdigitated with two intensity-modulated brachytherapy (IMBT) HDR boosts. EBRT was delivered via linear accelerator with 18 MV. The conformal isocentric four-field technique included the prostate, seminal vesicles, and up to the iliac chain. The daily doses were of 200 cGy to a total of 4,300 cGy in 23 fractions. The brachytherapy fractions were given on the 5th and 15th day of a 25-fraction hypofractionated treatment course following the Martinez protocol developed at William Beaumont Hospital in Royal Oak, Michigan (35–37). The day the patients underwent brachytherapy, no external dose was delivered. The brachytherapy clinical target volume was determined by intraoperative online transrectal ultrasound (TRUS), contouring the prostate capsule from base to apex and medial half of the seminal vesicles. A 5-mm expansion was given for the PTV. The HDR boost dose per fraction was 1,150 cGy to cover the PTV. The corresponding dosimetric values in terms of the mean dose are reported. Since December 2004 (with institutional review board approval), a modification of the prostate HDR boost protocol was started at Asturias Hospital. For these patients, it consisted of HA injected into the perirectal fat at the time of the second IMBT-HDR brachytherapy procedure, but just before delivering the dose. Treatment planning dosimetry and optimization was obtained intraoperatively and approved by the physician before the injection of HA and was delivered as planned after the injection. The treatment schedule for this protocol is depicted in Table 1.

Table 1. Treatment program

Weeks	External beam radiotherapy	Brachytherapy
First week	2 Gy/day, days 1–4	Fifth day, first HDR (1,150 cGy) + TLD no HA-I
Second week	2 Gy/day, days 6–10	No
Third week	2 Gy/day, days 11–14	15 day, second HDR (1150 cGy) + TLD + HA-I
Fourth week	2 Gy/day, days 16–20	No
Fifth week	2 Gy/day days 21–25	No
Total dose	46 Gy in 23 fractions	2,300 cGy in 2 fractions

Abbreviations: TLD = thermoluminescence dosimeter; HA-I = hyaluronic acid injection.

First IMBT: Having finished the HDR brachytherapy needle placement and before dose delivery, 10 thermoluminescent dosimeter (TLD) chips are placed within the prostatic urethra via a three-way catheter as well as in a rectal catheter. These catheters have been specially designed to measure delivered dose within the anterior rectal wall adjacent to the prostate and prostatic urethra. Intraoperative fluoroscopy is used for verification of the TLD's placement. In this fashion, we measure the delivered dose to both critical organs during the first IMBT-HDR treatment *in vivo*.

Second IMBT: During the second brachytherapy treatment (fraction 15th), the process is repeated as in the first treatment in terms of needle, urethral, and rectal TLD catheter placement. However, the injection of HA is performed just before the TLD catheter placement. Again, *in vivo* measurement of delivered dose to both rectum and urethra is obtained for dose delivered comparison between the two fractions as well as comparison with calculated doses.

Monotherapy

For those patients with favorable presentations, HDR or LDR brachytherapy with permanent seeds are used as curative treatments. The injection of HA is performed before needle placement. Because this work was started very recently, the results will be reported separately in a different publication after enough cases are performed for statistical analysis.

Fig. 1. The needle tip is placed in the perirectal fat.

Fig. 3. Rectal catheter with the thermoluminescence diameters are placed in the rectum.

Technique of hyaluronic acid injection

Whether the brachytherapy treatment with IMBT using HDR or permanent seeds are used as a boost or as monotherapy, the injection technique of HA in the perirectal fat is the same and occurs after all needles are in treatment position.

Step 1: The TRUS probe with the transperineal template is placed and fixed in the standard fashion. All treatment needles are placed under TRUS guidance.

Step 2: Using TRUS guidance, an additional needle is placed (Fig. 1) guiding the needle tip into the perirectal fat, between the posterior prostate capsule and the anterior rectal wall, at the level of the maximum transverse diameter of the prostate (reference level). Under direct TRUS guidance, the needle tip is advanced to the level of the seminal vesicles. Extreme care is taken not to perforate the prostate capsule with the needle tip and to keep the tip as far as possible from it without getting into the rectum.

Step 3: The additional needle is connected to the syringe containing 10 mL of HA. After aspirating to ensure that contact with a vessel had not occurred, between 3 and 7 mL of HA is injected within the perirectal space between the seminal vesicles and the apex of the prostate. This is performed under continuous TRUS guidance to view and verify the new space created by the injection. The total injected amount is related to the need for systematically creating a minimum of a 1.5-cm space between the prostate, seminal vesicles and rectum throughout this length.

Step 4: Under fluoroscopic guidance, the urethral (Fig. 2) and rectal (Fig. 3) catheters with the TLDs are placed. The TLD chips are placed longitudinally between the plane of the bladder neck and the apex of the prostate plane.

Step 5: After the IMBT-HDR treatment is completed, both catheters containing the TLD chips are removed for reading and comparison of dose measured with the ones placed during the first fraction.

Fig. 2. Urethral catheter with thermoluminescence dosimeters are positioned.

Hyaluronic acid

HA has an absorption coefficient equal to water. It is a polysaccharide found in human tissues as a component of the connective tissue. Normally, it plays a vital role in the skin and in the synovial fluid of the joints. It is normally degradable by the enzymatic system in a relatively short time. However, to make it last for months when used for the treatment of skin wrinkles and osteoarthritis, the compound is modified, making it stable for a duration close to 1 year before being reabsorbed by the body (38, 39). Two hyaluronic acid products are available in the United States: naturally occurring hyaluronan (Hyalgan; Sanofi-Aventis, Bridgewater, NJ) and synthetic hylan G-F 20 (Synvisc; Genzyme Corporation, Cambridge, MA). Hyalins are cross-linked hyaluronic acids, which gives them a higher molecular weight and increased elastoviscous properties. The pathologic changes of synovial fluid hyaluronic acid, with its decreased molecular weight and concentration, led to the concept of viscosupplementation. Several randomized trials have demonstrated the advantage of hyaluronic acid intra-articular injection when compared with placebo (40–42) or oral Naproxen (43, 44).

RESULTS

This is the first report in the medical literature using HA in oncologic treatments. We selected prostate cancer treatments with radiation as the first site to test this compound and its possible benefits.

Since 1998, Hospital Universitario Central de Asturias has been performing IMBT-HDR prostate boost brachytherapy following the Martinez (6, 9, 35–37) protocol. More than 330 patients were treated. The currently reported technique of injecting the HA in December 2004. We enrolled 27 patients in the Human Investigation and Ethical Com-

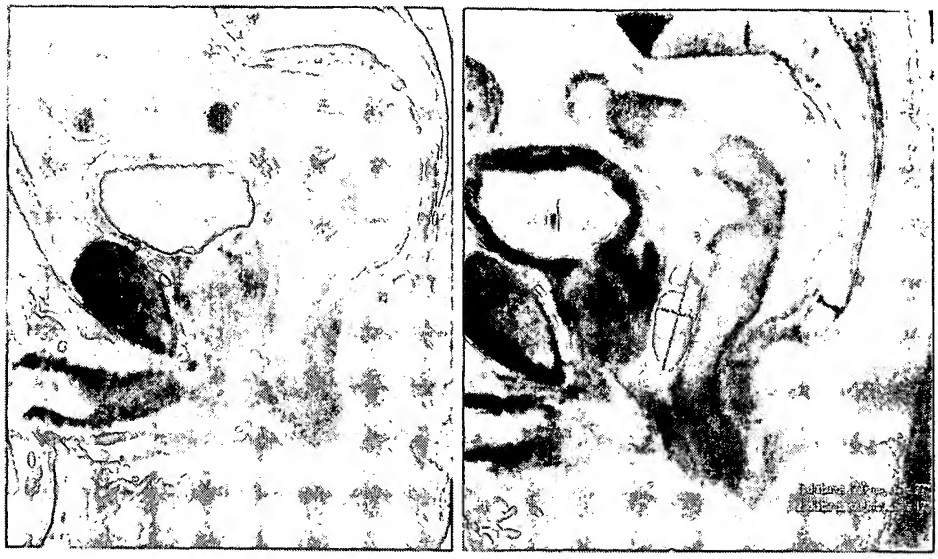

Fig. 4. Magnetic resonance image demonstrating the additional perirectal space created by the hyaluronic acid injection.

mitte-approved study. The mean follow-up is 13 months with a range of 9–22 months.

Patient characteristics

More than half of the patients (63%) presented with Stage ≥T3a, 26% as T2b, and 11% as T2a. The Gleason grade was <7 in 41% of the patients and ≥7 in the remaining 59%. The median prostate-specific antigen value was 21 ng/mL with a range of 8–7 ng/mL. The median prostate volume implanted was 38 mL (range, 16–69 mL). The median patient age was 67 years (range, 55–77 years).

Radiologic studies

Figures 4 and 5 illustrate the patients' X-rays before and after HA injection, demonstrating the newly created space between the prostate capsule and the anterior rectal wall. Figure 4 corresponds to a magnetic resonance image (MRI) before and after the injection showing at least an additional 2 cm perirectal space through out the prostate length in the longitudinal plane from the seminal vesicles to the prostatic apex. Figure 5 depicts a TRUS image of an IMBT-HDR boost patient demonstrating the additional perirectal space created by the HA injection.

TLD Analysis

The dose delivered to the rectum and urethra using TLDs was measured. The mean number of TLD chips was 10, covering a length of 6 cm in each site and for each fraction. The concordance between the urethra measured and calculated dose was between −1.1% and 5.1%. For the rectum, the range was wider due to the anatomic distortion produced by leaving the probe inside the rectum during TRUS image

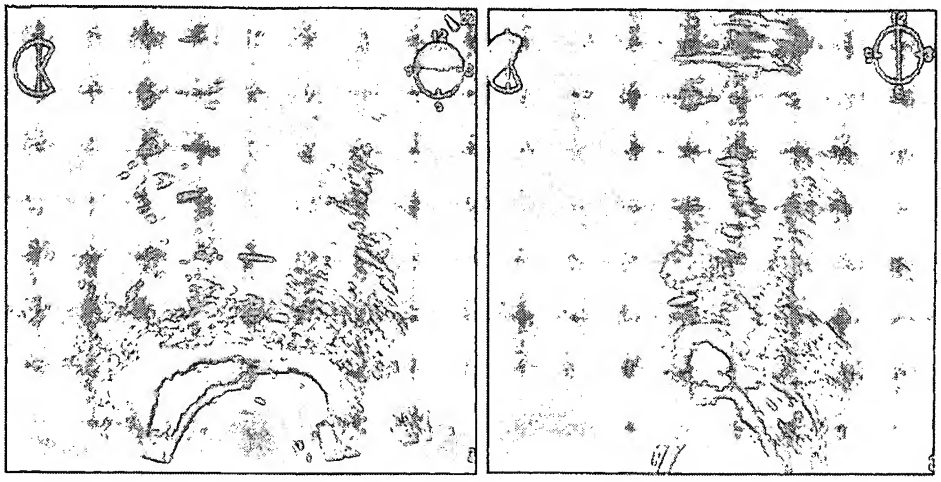

Fig. 5. Transrectal ultrasound image demonstrating the additional perirectal space created by the hyaluronic acid injection.

Table 2. Difference in the mean rectal dose with and without hyaluronic acid injection

	Without hyaluronic acid	With hyaluronic acid	*p* values
Patients	27	27	
Mean	47.1%	39.2%	< 0.001
Standard deviation	9.7%	6.2%	
Rectum mean Dmax	708.1cGy (± 135.2 SD)	507.4 cGy (± 113.5 SD)	< 0.001
Rectum mean Dmean	608.3 cGy (± 114.6 SD)	441.8 cGy (± 1.3.2 SD)	< 0.001

capture and intraoperative dosimetry. Removing the probe before delivering treatment induced anatomic distortion and increased the distance to the rectum. In such cases, the calculated rectal dose was the worst-case scenario. On the other hand, the calculated rectal dose using postimplant CT did not accommodate the probe displacing the anterior rectal wall closer to the Ir-192 seed.

For this analysis, the mean rectal dose is normalized with respect to the mean urethral dose, expressing the results as a percentage difference between the two measured doses. The initial mean measured dose, when compared with the doses measured after HA injection, was lower. The mean value measured with HA was 39.2%; the mean value without HA was 47.1% (SD 9.7% and 6.2%, respectively); $p < 0.001$. The rectum mean Dmax was 708.1 cGy (± 135.2 SD) without HA and 507.4 cGy (± 113.5 SD) after injection; $p < 0.001$. The rectum mean Dmean was 608.3 (± 114.6 SD) preinjection and 441.8 cGy (± 103.2 SD) after injection ($p < 0.001$) when an HDR dose of 1,150 cGy was delivered to the prostatic PTV (Table 2).

To assess HDR implant quality, the dose and volume mean doses were examined. The mean V100 was 94.4% (range, 90.7–97.6%). The mean V90 was 98.3 (range, 96.9–99.7%). The HDR brachytherapy prescribed dose was 1150 cGy to the PTV. The mean D90 was 1213 cGy (range, 1159 cGy–1268 cGy. Table 3 highlights a more complete dose–volume analysis. In addition, the relative difference (%) between the maximum TLD measured dose in the urethra and the maximum calculated dose by the treatment planning computer was analyzed. It appears that the measured dose is slightly higher than the calculated one. However, the SD of 11% is rather large. Figure 6 depicts the distribution of 54 applications in 27 patients.

Table 3. High-dose-rate prostate brachytherapy boost dose and volume mean values

Mean V90 (max – min)	98.3% (96.6–99.7%)
Mean V100 (max – min)	94.4% (90.7–97.6%)
Mean V150 (max – min)	20.6% (17.3–31.3%)
Mean V200 (max – min)	6.1% (4.2–9.0%)
Mean D90 cGy (max – min)*	1,213 cGy (1,159–1,268 cGy)

* Prescribed dose was 1,150 cGy to cover the transurethral ultrasound–based intraoperative planning target volume.

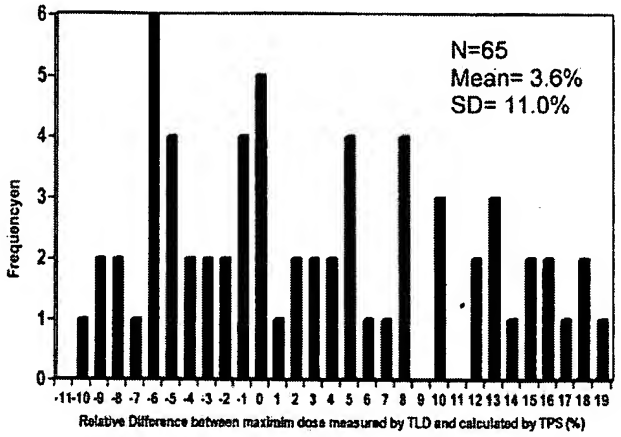

Fig. 6. Urethra maximum dose.

Tolerance

We have not seen side effects related to the injection or the compound itself. There is no toxicity in the fat tissue or in rectal function. Patients have not complained of pain, tenesmus, rectal pressure, or sensation of rectal filling. During the posttreatment follow-up evaluation, no patients complained of difficulty with defecation that could be attributed to the HA injection. All patients had a TRUS evaluation 9 months' postinjection/implant. No changes in the volume or distance between the prostate capsule and the anterior rectal wall were noted. Additional follow-up with CT and MRI confirms the same information obtained by TRUS. However, decreases of the newly created space at or after 1 year have been observed.

DISCUSSION

The relationship between the probability of tumor control and dose has long been established. In prostate cancer treatment, better results have been reported with increasing radiation doses (9). However, the increase in radiation dose to the prostate has also has an impact of higher probability of toxicity, particularly in the rectum (38). Few dosimetric planning exercises have been tested to decrease rectal toxicity from higher prostatic radiation doses. Additionally, prostate fixation devices, rectal balloon tubes (45, 46), and dietetic approaches (47) have been tested to decrease the inaccuracies of dose delivered to the prostate and rectum. Although the rectal balloon tube was designed as an immobilization device for the prostate, Wachter *et al.* (45) reported statistically significant ($p = 0.04$) less rectal filling variations for those patients with the balloon vs. without. The balloon led to a reduction in partial posterior rectal wall in patients whose prostate was treated alone. However, when the seminal vesicles were treated, this advantage was lost. Asturias and William Beaumont Hospital developed a technique that appears to be effective in keeping both the prostate and seminal vesicles away from the ionizing radiation. In addition, when compared with the daily balloon placement technique, our approach eliminates patient dis-

tress associated with having a balloon inserted into the rectum daily as well as the inaccuracies associated with the placement.

During the initial discussions necessary to develop this protocol, we considered at length the wisdom of an injection of HA in the vicinity of the prostate. Elevated HA levels in prostate tumor-associated stroma have been shown to carry a worse prognosis. Our contention was that the HA is never injected in the prostatic stroma, but placed into the perirectal fat, with the expectation that the prostate capsule act as a barrier. In addition, because the HA is absorbed locally by the lipocytes, it does not leak into the prostatic stroma. For these reasons, we felt it was safe to use.

More recently, three-dimensional planning techniques with and without rectal blocking during the boost and intensity modulated beams have been used to minimize rectal damage. Longer follow-up is needed to determine if there is a benefit from these planning exercises. Since 1996, at Beaumont, we pioneered image-guided radiotherapy with offline correction using the adaptive radiotherapy process (ART) (48–51). Cognitive of the significant intra- and intertreatment prostate and normal tissue motion and deformation occurring during the 7–8 weeks of a prostate cancer radiotherapy treatment, we developed the ART process. ART corrects for any systematic components of prostate and rectal motion and deformation and designs a margin, which is patient specific to account for both random components and potential residual systematic components (50, 51). We were able to demonstrate a reduction of PTV of 24% (50). In addition, by applying the ART process, namely developing a patient-specific PTV to prostate cancer patients, significant dose escalation was achieved without increasing either genitourinary or gastrointestinal toxicity (51). We have published our results as it relates to rectal tolerance after treatment of prostate cancer with adaptive image-guided radiotherapy. Vargas *et al.* (52) recently reported a dose–volume analysis of predictors for chronic rectal toxicity with our ART dose escalation study. If the rectal DVH is used to select the prostatic dose level, the risk of chronic toxicity is independent of the dose delivered to the PTV. Vargas also found that patients experiencing acute rectal toxicity are more likely to experience chronic toxicity. Consequently, when our dose constraints were met, rectal toxicity was independent of total prostatic dose using our adaptive therapy technique; however, Grade 1-2 chronic toxicity was seen in >15% of the patients, providing an opportunity for improvement. During the last 2 years, in our daily practice, we introduced online correction with cone-beam guidance using the "Elekta Synergy" machine developed at Beaumont. Longer follow-up is required to assess the benefit from online cone-beam image guidance. Although daily setup errors and internal organ motion with deformation are significant problems for daily EBRT, they are irrelevant for HDR brachytherapy. During the 10- to 18-min treatment time, during which the prostate is fixed by 15–18 needles, all these issues disappear.

The mechanism for postradiation rectal injury leading to symptoms of proctitis, bleeding, and rectal ulcers are related to the damage of the small vessels and arterioles in the rectal cryptae and subsequent development of edema and fibrosis of the intraluminal cryptae of the rectal mucosa (29, 53). The consequence is the increased fragility of the rectal mucosa with formation of telangiectatic vessels, which have a tendency to bleed and ulcerate.

In 1996, Wallner *et al.* (21) were one of the first groups postulating guidelines to minimize both urethral and rectal morbidity after transperineal Iodine 125 seed prostate implantation. They presented a correlation of increasing rectal mucosal dose with increased rectal toxicity. They recommended to keep the rectal dose ≤100 Gy and to minimize the volume of rectum receiving high doses. Later, in 1999, Merrick *et al.* (28) reported their rectal dose analysis following LDR prostate brachytherapy with Iodine 125 and Palladium 103 seeds. In the anterior rectal mucosa, dose averages to about 85% of the prescribed dose, regardless of isotope used, and the length of the anterior rectal mucosa receives 100% and 120% of the prescribed dose at approximately 10 mm and 5 mm. Respectively, the incidence of mild self-limited proctitis will be in the range of approximately 9% without incidence of rectal ulceration or fistula formation. We are fairly certain that with the additional newly formed space of 2 cm by the injection of HA, these constraints could easily be met, hence decreasing further the probability of rectal toxicity.

From the EBRT experience, increasing prostate doses similarly increases rectal toxicity. Teshima *et al.* (38) reported on rectal bleeding after conformal three-dimensional treatment of prostate cancer: time to occurrence, response to treatment, and duration of morbidity. The mean duration for Grade 3 bleeding was 10 months; 75% of patients responded to medication and multiple local coagulation treatments. Nonresponders continued to bleed up to 35 months after the third coagulation. In their multivariate analysis, dose was the only significant factor associated with Grade 2 ($p = 0.01$) or Grade 3 ($p = 0.01$) rectal bleeding. They concluded that chronic rectal bleeding is sequelae of high-dose conformal treatment of prostate cancer. Appropriate shielding of the rectal mucosa limiting the dose to <72 Gy is required to avoid a high incidence of these complications. Of course, the concern is that blocking the rectal mucosa for patients with intermediate and high-risk prostate cancer might also block the tumor because of the uncertainties described previously. On the other hand, based on our results obtained with HA injection demonstrating a significant impact on rectal mean Dmax dose and rectal mean Dmean dose, the extra space created by the HA injection could facilitate keeping the rectal dose below 70 Gy throughout the entire length of the rectum between the seminal vesicals and the apex of the prostate.

Considering these reports on postradiation rectal toxicity and our own extensive experience at Beaumont with all types of radiation used for the treatment of prostate cancer, we began (at the University of Asturias in Spain) the study presented here in an effort to keep the same outcome success from HDR and

pelvic EBRT (6, 8–10, 36, 37) to further minimize rectal toxicity by creating a real and stable space between the prostate and rectum. According to our data (Table 2), the increase in distance (mean 2 cm along the length of the prostate) between rectum and posterior prostatic capsule created by the perirectal injection of HA, an inert nontoxic compound, is enough to provide a significant radiation dose reduction from either brachytherapy (LDR or HDR) or for high-dose image-guided EBRT. The significant reduction in rectal Dmax and Dmean will be particularly beneficial in hypofractionated IMRT-image guided prostate cancer treatments.

We will be testing the same concept of dose reduction to critical structures in other sites where the injection of HA is feasible; certainly in gynecologic malignancies and preoperative colorectal and sarcoma radiation treatments.

Longer follow-up is needed to assess the true gain in rectal toxicities for these EBRT and HDR boost patients. We are conducting a HIC-approved protocol; a randomized trial using LDR permanent seeds or HDR as monotherapy with or without HA injection. Another open protocol is hypofractionated EBRT using image guidance with online cone beam and IMRT planning. For this study, the implantation of three intraprostatic markers, 4 cm in length visicoils, is performed before MRI-based planning and virtual simulation. We are proposing the approval of HA injection at the time of TRUS-guided visicoils placement before the patient's virtual simulation. The three implanted visicoils will assist with image guidance, whereas the goal of the HA injection is to decrease rectal toxicity from higher daily doses.

CONCLUSIONS

We are reporting a noble technique to decrease rectal toxicity and improve quality of life for patients with prostate cancer undergoing any form of radiotherapy. When HA is injected (under TRUS guidance) transperineally into the anterior perirectal fat, one can demonstrate by TRUS, CT, and MRI the development of a new space between the posterior prostate capsule and the anterior rectal wall. This newly created space has a mean value of 2.0 cm in the anteroposterior dimension throughout the length of the seminal vesicles and prostate gland and remains stable for up to 1 year. TLD measurements demonstrated a statistically significant reduction in mean Dmax after HA injection from 708 cGy to 507 cGy ($p < 0.001$) and a mean Dmean drop from 608 cGy to 442 cGy ($p < 0.001$) after injection when an HDR boost dose of 1,150 cGy was delivered to the prostatic PTV. Longer follow-up is necessary to demonstrate the real gain with decreased rectal toxicity. This also opens the possibility of using this methodology in the treatment of other malignancies whereby adjacent normal tissues may prevent the delivery of high doses of ionizing radiation for tumor control.

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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	Attorney Docket No.: 3516.32US01
Noyes	Confirmation No.: 1769
Application No.: 10/602,526	Examiner: Sheikh, H.
Filed: June 24, 2003	Group Art Unit: 1618

For: FILLERS AND METHODS FOR DISPLACING TISSUES TO IMPROVE RADIOLOGICAL OUTCOMES

DECLARATION OF

Amarpreet Sawhney

UNDER 37 C.F.R. § 1.132

This Declaration is submitted to the Unites States Patent and Trademark Office to provide information relevant to a patent application for which I have an interest as disclosed below.

1. I, Amarpreet Sawhney, am an adult residing in these United States. I am a partner in Incept, LLC and CEO of Augmenix, Inc., which are, respectively, the assignee and an exclusive licensee in a field of use of the above-referenced patent application (the Application).

2. I am an expert with respect to materials used in situ in the body by virtue of my training and work with these materials for over ten years, including development of photopolymerizable sealants and in situ polymerized hydrogels. A partial curriculum vitae is attached.

3. I have read the Application. In its working examples, it describes a treatment of introducing a suitable filler between a rectum and prostate in humans to displace surrounding tissues for purposes of radiation therapy. Many fillers are described in the Application that can be also be used, as is evident from the fact that collagen was actually tested and many fillers have the relevant properties of being biocompatible, biodegradable, and introducible in a flowable state (for example, via a syringe needle or

catheter) to form a material that can displace tissues relative to each other and also stay in the intended location.

4. Scientists and engineers that read the Application can readily appreciate how to obtain, make, or use various fillers as taught therein. I review some of them below:

a. Collagen

Collagen is suited to the treatment because it is biocompatible, biodegradable and can be introduced in a flowable state (for example, via a syringe needle or catheter) to form a material that can displace tissues relative to each other and also stay in the intended location.

Collagen forms a solid hydrogel material after it is allowed to react with itself in situ in response to a change of temperature and/or pH. This self-reaction is a crosslinking process. Other fillers can be similarly used, with the crosslinks being formed by covalent, ionic or hydrogen bonds. The publication "Autologous Collagen Dispersion (Autologen) as a Dermal Filler", *Arch. Facial Plast. Surg*, Jan-Mar: 48-52 (2000) by Sclafani et al., reviews some of the history of fillers, and collagens used as fillers, including those sold under the brand names of AUTOLOGEN, ZYPLAST, and DERMALOGEN.

b. Hyaluronic Acid

Hyaluronic acid may be used for the treatment. Hyaluronic acid is biocompatible, biodegradable, and can be introduced in a flowable state to form a material to displace tissues and stay in the intended location. The publication "Transperineal Injection of Hyaluronic Acid. . . ", I. J. Radiation Oncology, 69(1):95-102 (2007) by Prada et al. describes injection of hyaluronic acid into an intended location between the rectum and prostate. This published report demonstrates that hyaluronic acid was successfully used. Its authors note that hyaluronic acid is commercially available, e.g., as HYALGAN or G-F 20. It has numerous ionic side groups that interact with each other to form a viscous, cohesive mass, and its side chains can be modified to provide a wide range of physical properties. Hyaluronic acid is a polysaccharide.

c. Polysaccharides

Polysaccharides may be used for the treatment. Polysaccharides are relatively complex carbohydrates that are polymers made up of many monosaccharides joined together by glycosidic bonds. They are generally very large macromolecules. They tend to be amorphous and insoluble in water. Other polysaccharides are, for instance, cellulose, chitin, dermatan sulfate, alginate, and chondroitin sulfate. These may also be prepared to form a cohesive mass, and its side chains can be modified to provide a wide range of physical properties. Alginate may be crosslinked in situ, for example, by exposure to a divalent cation such as calcium. And Prada et al. described, for instance, successful results using hyaluronic acid. As with hyaluronic acid and collagen, many

polysaccharides share common physical and chemical characteristics that make them generally suited for use as fillers in the treatment by preparing them to form crosslinks.

d. Fibrin or Fibrinogen

Fibrin or fibrinogen may be used for the treatment. Fibrinogen is a natural monomer that can be polymerized into a fibrin clot. Fibrin glues rely on this principle to make materials in situ as needed. Fibrin clots are biocompatible, biodegradable, and can be introduced in a flowable state to form a material to displace tissues and stay in the intended location. The publication "The Use of Fibrin Glue in the Surgical Operations", Acta Bio Medica 74:Suppl. 2:21-25 (2003) by Canonico reviews the use of fibrin glue in surgeries. A commercial source of fibrin glue is TISSUCOL. Many delivery devices for making the clot at an intended site are known, e.g., as in U.S. Pat. No. 4,874,368.

e. Albumin or gelatin.

Albumin or gelatin may be used for the treatment. Schemes similar to those used for fibrin glues have been used to crosslink albumin or gelatin to make a biocompatible, biodegradable, material that can be introduced in a flowable state to form a material to displace tissues and stay in the intended location. For instance, the publication "Feasibility Study of NeoMend. . . ", AJR, 180:533-538 (2003) by Funovics et al. describes a crosslinked albumin material made by NEOMEND and delivery device, see also U.S. Pat. No. 7,247,314 to NEOMEND. U.S. Pat. No. 5,618,551 describes a crosslinked gelatin or collagen filler.

f. Polyethylene glycol

Polyethylene glycol may be used for the treatment. For instance, the article entitled "DuraSeal MR and CT Imaging. . . ", Kacher et al., reports on studies performed by Harvard Medical School using DURASEAL synthetic hydrogel, a biodegradable polyethylene-glycol based material that transforms from liquid to solid in situ. The material was used in the dura. Follow-up imaging showed that the material remained in place as intended.

The attached "Polyethylene Glycol Materials" report describes experiments performed under my direction. This report has been electronically redacted to remove trade names and dates. These experiments describe deposition of a polyethylene-glycol based material that transforms from liquid to solid in situ, as placed in six domestic swine. The data shows that the material is present at both the 2 and 6 month time points and there is no adverse response to the material being present for these durations. The material was placed into the *rete mirabile*, which is a model for arteriovenous malformation.

The material was a polyethylene glycol diacrylate polymerized by an initiator (Fe-gluconate or H_2O_2). The acrylates polymerize with each other in response to exposure to the initiator and form a crosslinked material. These were injected and formed a firm material that stayed into place, as evidenced at 2 and 6 months by direct observation after sacrifice and histology. This particular material is not biodegradable but can be made biodegradable with suitable linkages as needed, for instance by reference to U.S. Pat. No. 5,410,016. The fact that this material formed in situ and stayed in place in the

vasculature of the *rete mirabile* shows that polyethylene glycol materials will be effective for the treatment in the Application.

The Application also describes a solution of polyacrylic acid and polyethylene oxide that can be used, with these materials forming a gel when mixed. The Application also describes in situ polymerizable biodegradable photopolymerizable polyethylene glycols (see reference to WO 93/17669) and specifically mentions polyethlyeneglycol-oligolactylacrylates.

g. Thixotropic polymers or thermoreversible polymers.

Polysaccharides can be prepared as thixotropic materials, meaning materials that have a significantly lower viscosity when flowing as compared to not flowing. For example, hyaluronic acid can be prepared as a thixotropic composition, as described in the Application. Thermoreversible polymers, also described in the Application, e.g., PLURONICS or TETRONICS which are crosslinked by hydrogen bonding and/or by a temperature change may be used. Artisans can readily obtain these materials from commercial sources. The suitability of hyaluronic acid has already been described. PLURONICS and TETRONICS materials are well known to form gels and may be crosslinked if needed to stay in place as needed.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____

Amarpreet Sawhney

Date: 8/14/08

NOTARY:

Roberta M. Sawyer

ROBERTA M. SAWYER

Expires 7/19/13

AMARPREET SINGH SAWHNEY

Lexington, MA 02421

I-Therapeutix/Augmenix, Inc.
204 2nd Ave.
Waltham, MA 02451

EXPERIENCE:

Aug' 06 - Present: **President, and CEO, I-Therapeutix, Inc.:** Responsible for creation of company that is developing sealant and drug delivery products for ophthalmic applications.

Jan' 08 - Present: **President, and CEO, Augmenix, Inc.:** Responsible for creation of company that is developing biomaterials for minimizing radiation side effects in prostate and breast cancer therapy.

July '98- Aug' 06: **Founder, President, and CEO, Confluent Surgical, Inc.:** Responsible for creation of company that is developing several innovative biosurgery products. SprayGel, the world's first synthetic post surgical adhesion barrier to be commercialized, and DuraSeal, the only approved surgical sealant for Neurosurgery. Raised $60MM for Confluent and business sold to Tyco Healthcare for $245MM.

Aug '92- Dec '96: **Director and Technology Founder, Focal Inc.:** Co-inventor of core technology. Headed development of and created the world's first synthetic sealant, FocalSeal, from concept to commercialization. Responsible for all new technology initiatives and new product development. Focal's scientific spokesperson for all clinical, regulatory, financial, and scientific audiences. Focal went public and was acquired by Genzyme Corp.

OTHER APPOINTMENTS and AWARDS:

Chairman and Board Member – marketRx Inc.: A Bridgewater, NJ based company developing marketing automation and intelligence software, tools, and consulting services for the pharmaceutical industry.

Board Member – Access Closure, Inc.: A Palo Alto, CA based company developing therapies targeted to the management of vascular access for interventional radiology and cardiology applications.

Board Member – Square One Inc.: A Palo Alto, CA based company ostial and bifurcation stent technologies for interventional radiology and cardiology applications.

Board Member – Sotiera, Inc.: A Natick, MA based company developing minimally invasive vertebroplasty systems.

General Partner – Incept, LLC: A Lexington, MA based company that serves as an enabler for healthcare entrepreneurial efforts.

Board Member – TiE Boston: The New England chapter of a worldwide non-profit organization dedicated to fostering entrepreneurship.

Mass High Tech All Star Award 2007

University of Texas at Austin "Outstanding Young Engineering Graduate Award" 2007

Winner of Ernst and Young Entrepreneur of the Year award for New England, 2006.

DuraSeal recognized as top 3 most significant approvals by FDA for 2005 and Most Innovative Product by Frost and Sullivan, 2005.

Winner of Global Indus Technovators Award by MIT, 2003.

EDUCATION: University of Texas at Austin- Ph.D. in Chemical Engineering. Graduated August '92. Dissertation Title: Biocompatible microspheres and microcapsules for animal tissue encapsulation and transplantation.

University of Texas at Austin- M.S. in Chemical Engineering. Graduated May '89. Thesis Title: Biodegradable polymers for the prevention of postoperative adhesions.

Indian Institute of Technology, Delhi- Bachelor of Technology in Chemical Engineering. Graduated May '87. Thesis Title: Design of a reverse osmosis plant for the treatment of sugar mill effluents.

PATENTS **More than about 45 issued**

PUBLICATIONS **More than about 20 peer-reviewed**

INVITED AND CONFERENCE PRESENTATIONS **More than about 50**

GUEST REVIEWER:

1. Biomaterials
2. Journal of Biomedical Materials Research
3. Journal of Biomaterials Science
4. Biotechnology and Bioengineering

United States Patent [19]

Miller et al.

[11] **Patent Number: 4,874,368**

[45] **Date of Patent: Oct. 17, 1989**

[54] **FIBRIN GLUE DELIVERY SYSTEM**

[75] Inventors: **Curtis H. Miller**, Burnsville, Minn.; **I. Kaufman Arenberg**; **John H. Altshuler**, both of Englewood, Colo.

[73] Assignee: **Micromedics, Inc.**, St. Paul, Minn.

[21] Appl. No.: **224,078**

[22] Filed: **Jul. 25, 1988**

[51] **Int. Cl.**[4] **A61M 5/08**

[52] **U.S. Cl.** **604/82**; 222/137; 604/191

[58] **Field of Search** 604/82, 191; 222/129, 222/137, 145

[56] **References Cited**

U.S. PATENT DOCUMENTS

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4,735,616	4/1988	Eibl et al.	604/191

FOREIGN PATENT DOCUMENTS

25913	2/1884	Fed. Rep. of Germany	604/191

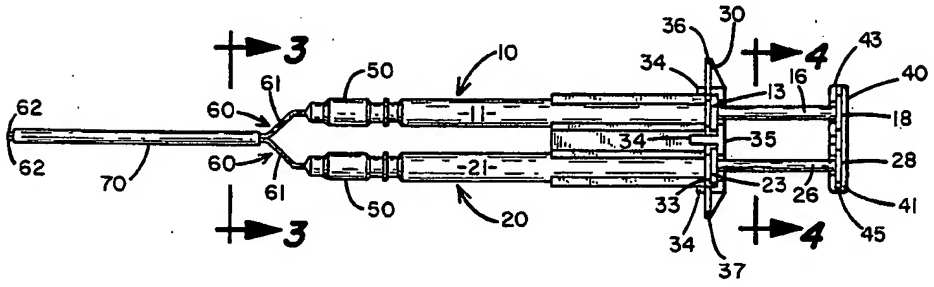
Primary Examiner—Dalton L. Truluck

Attorney, Agent, or Firm—Orrin M. Haugen; Thomas J. Nikolai; Frederick W. Niebuhr

[57] **ABSTRACT**

An improved fibrin glue delivery system is disclosed. The delivery system is comprised of a pair of syringe tubes which can be actuated by plungers simultaneously or independently, a connecting member which holds the syringe tubes in parallel alignment and a unique needle assembly which ensures the components in the syringe bodies will not be comingled until they reach the treatment site. The unique needle assembly also permits the user to manipulate the needles to enhance visibility when the surgeon is working through a speculum or when direct access is difficult.

3 Claims, 1 Drawing Sheet



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□□□□ Experimental Report

Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□

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Keywords:

Polyethylene Glycol Materials Report

1 Executive Summary

This experimental report describes the chronic deposition of the □□□□ Surgical Liquid Embolic material in the *rete mirabile* (RMB) of six domestic swine, three each on □□□ and □□□ at □□□. The data shows that the material is present at both the 2 and 6 month time points and there is no adverse response to the material being present within the vasculature for these durations.

2 Conclusions

- All six animals showed acute occlusion. (lack of flow past the treated RMB at t=5 minutes)
- None of the animals showed any signs of neurological deficit at any time.
- All six RMBs remained occluded at 2 and 6 months.
- Material was present after 2 and 6 month survival.
- Material does not cause any adverse inflammatory response.
- There was no observed embolization or polymerization of materials distal to the implant sites.
- Upon explant it was noted that the materials did not penetrate deeply into the rete, but merely blocked the entrance.
- At 2 months the histology report showed a classic chronic host response to the implant. (see Pathology report ANS 781)
- At 6 months the histology report showed a mild and normal foreign body vasculitis. (see Pathology report ANS 871 and notebook □□ p. □□□)

3 Objectives

- To determine the long-term tissue response and persistence of Liquid Embolic embolization of the RMB.
- To evaluate the potential of distal embolization or downstream polymerization.

4 Background/References

The *rete mirabile* is a well-established chronic model for neurovascular AVM. It has been identified as a suitable area to test the □□□□□ Liquid Embolic material for use as a treatment for neurovascular AVM.

Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□

5 Materials/Equipment

5.1 7F Introducer Sheath (Cordis, Inc.)
5.2 0.035" X 300cm Wholey Wire (Mallinckrodt, Inc.)
5.3 6F X 100cm Multi-Purpose Guide Catheter (ACS, Inc.)
5.4 0.014" X 300cm Std. Floppy II Guide Wire (ACS, Inc.)
5.5 1cc Luer Lock Syringes (See notebook □□ p. □□□)
5.6 1cc Syringe Holder Assembly (Micromedics, Inc.)
5.7 Liquid Embolic Delivery System (See notebook □□ p. □□□.)
5.8 Embolic Materials

Component A:	20% 3.4K PEG di-Acrylate, 20% Metrizamide, 1% Fe-gluconate. (MC-14-53)
Component B:	20% 3.4K PEG di-Acrylate, 20% Metrizamide, 3% of 5% H_2O_2. (MC-14-53)

6 Methods

6.1 The right RMB of each animal was accessed with a 0.035" guide wire and 6F guide catheter through a femoral access.
6.2 A pre-procedural arteriogram was performed confirming patency of the RMB and surrounding vasculature.
6.3 The delivery system was flushed with heparinized saline.
6.4 The delivery system was advanced, over the 0.014" guide wire to the area just proximal to the rete mirabile and the wire was removed.
6.5 Two 1cc syringes were loaded with material one syringe each with each component respectively, placed in the handles and attached to the catheter (Component A to the clear connector).
6.6 Using fluoroscopic guidance and digital subtraction, the Liquid Embolic was injected through the catheter, carefully "metering" the material in while watching for distal run-off from the RMB.
6.7 An arteriogram was performed confirming lack of flow to the RMB.
6.8 After five minutes, a second confirmatory arteriogram was performed.
6.9 Procedure complete, reversal of anesthesia and careful watching for 24 hours.
6.10 A total of six animals were enrolled in this study. Three each of these animals were survived for 2 and 6 months with interim diagnostic arteriograms at 2 and 6 weeks respectively. At sacrifice, the RMBs were harvested and sectioned for histological evaluation by a pathologist. The animals were evaluated for any overall gross effects in the kidneys, heart, liver and brain.

7 Results/Discussion

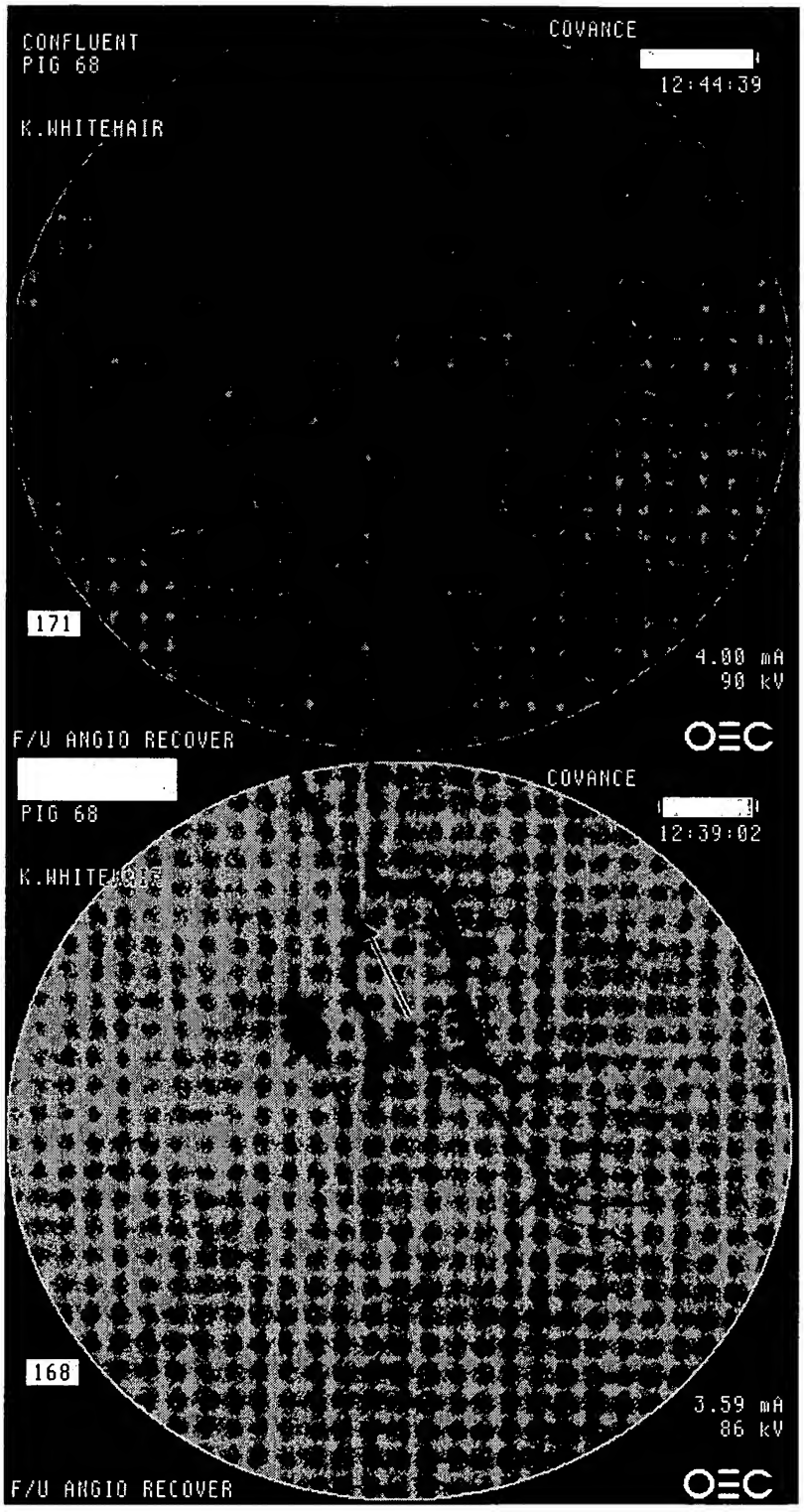
Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□

- Repeat injection was performed during the second procedure due to insufficient embolization after the initial injection.
- The catheters did not seem to have any gel stuck to it upon removal, nor were they clogged as was seen during the second injection of the second procedure.
- Guide-wire movement needs improvement.
- Guide-wire must be able to be back-loaded into the catheter.
- The delivery system needs a tip marker and not a radiopaque shaft.
- Material appeared to ooze from the distal catheter tip after removal from the animal and did not seem to polymerize.
- All six animals were successfully embolized as confirmed by arteriogram at t=5 minutes
- At 2 weeks (7/26/99), there was no flow into any of the treated vessels, with only slight crossover from the contralateral sides. (animals 333, 495 and 337 7/26/99). At 8 weeks (9/13/99), just prior to sacrifice, animals 333 and 337 showed no flow into the treated region and slight and almost no crossover from the contralateral sides respectively. Animal 495 at 6 weeks showed a slight blush of contrast into the treated RMB, which hadn't been noted previously. These animals were subsequently sacrificed and gross and histopathology was performed showing no adverse inflammatory response and no distal embolization or material polymerization to the brain.
- At 6 weeks (9/13/99), animals 513 and 496 showed no flow into the treated vessels and very slight crossover into the distal treated rete sides. Animal 68 showed an extremely slight blush of contrast into what appeared to be the treated RMB but was noted to be a small side branch that was not part of the RMB vasculature and there was only slight crossover from the contralateral side.
- At 24 weeks (12/21/99), animals 513, 496 and 68 all three RMBs remained occluded as evidenced by arteriogram with no contrast crossover from the contralateral carotid artery arteriogram. It was noted that since the animals had grown substantially from implantation, it was necessary to deviate from the protocol slightly and a 5F X 130cm long guide catheter was advanced inside the 6F guide catheter in order to reach the targeted vessel sites. These animals were subsequently sacrificed. Gross pathology and histopathology showed no adverse inflammatory response or non-targeted vessel embolization.

The following figures will show the still photos for the pre and post-procedural arteriograms.

Experimental Report

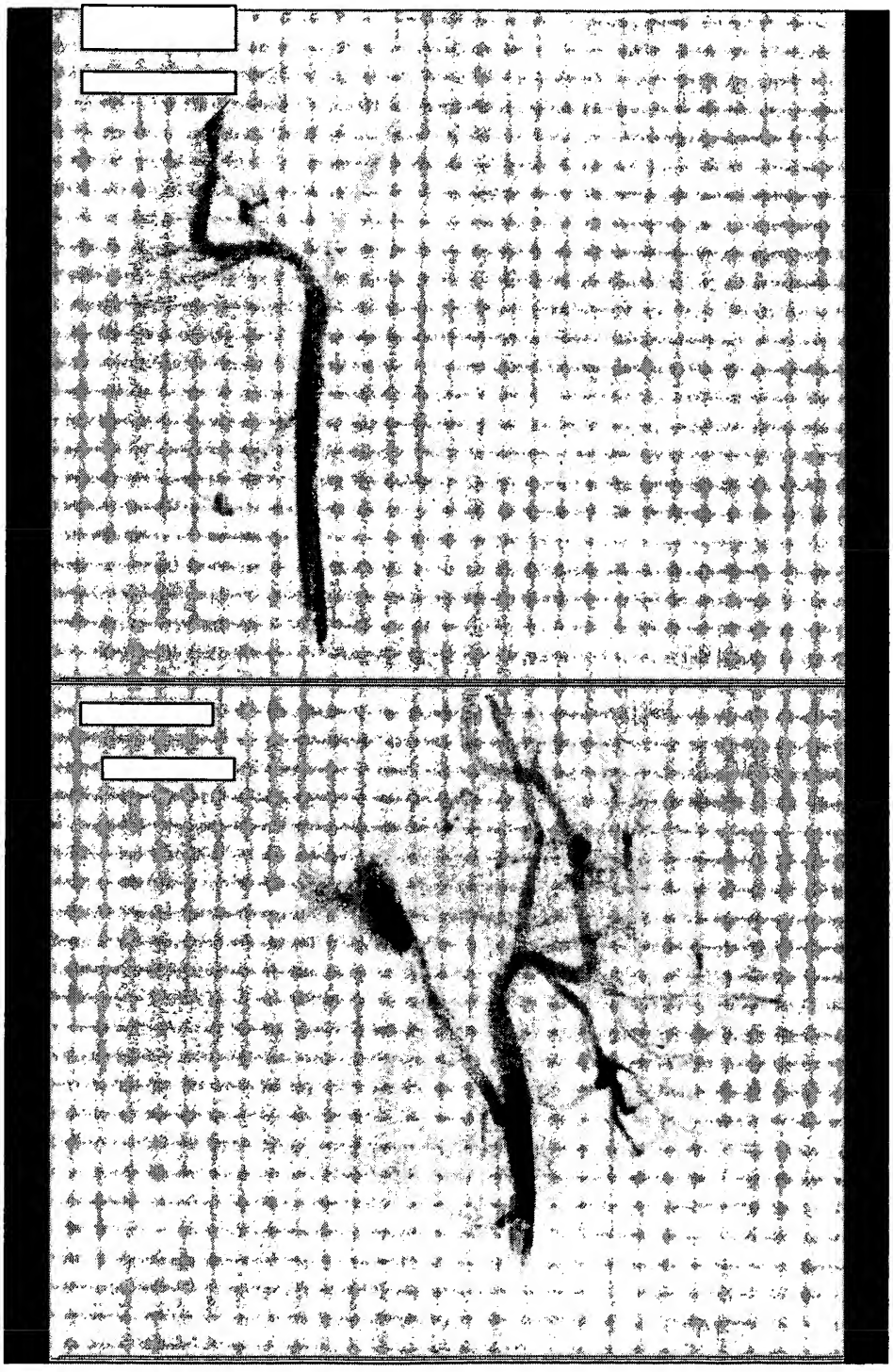
Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□



Ipsilateral arteriogram follow-up at six weeks (animal # 68)

Contralateral arteriogram follow-up at six weeks (animal # 513)

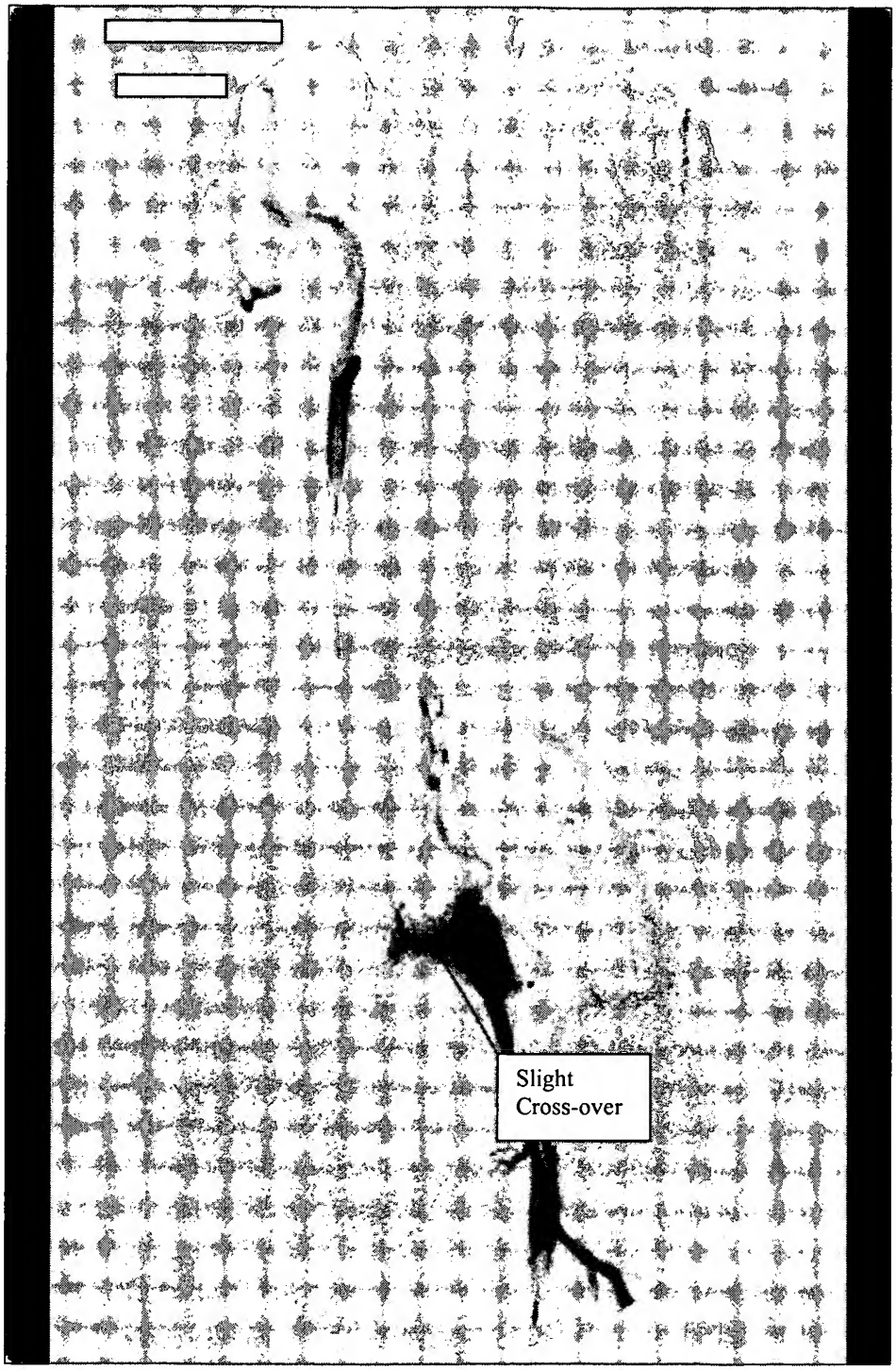
Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□



Ipsilateral final arteriogram at six months (animal # 68)

Contralateral final arteriogram at six months (animal # 68)

Title: Chronic Rete Mirabile Embolization	Date: □□□
Report Number: □□□	Prepared by: □□□



Ipsilateral final arteriogram at six months (animal # 513)

Contralateral final arteriogram at six months (animal # 496)

8 Completion

DuraSeal MR and CT Imaging: Evaluation in a Canine Craniotomy Model

Daniel F. Kacher*, MS, Kai Frerichs*, MD, Jeffrey Pettit†, AAS, RVT, Alexander M. Norbash*, MD

*Brigham and Women's Hospital, Harvard Medical School

†Harvard Center for Minimally Invasive Surgery, Harvard Medical School

SUMMARY: This report characterizes the MRI and CT appearance of DuraSeal Dural Sealant following implantation in a canine craniotomy model. Two canines received DuraSeal application to the dura following craniotomy. After recovery, animals underwent CT and MR imaging at 3 days, and at 2, 4, 6, 8 and 10 weeks. This document outlines the optimal imaging techniques for DuraSeal, and characterizes the hydrogel appearance over time using standard imaging protocols.

Introduction

The need for an effective, tissue compatible sealant for dural closure augmentation is widely recognized. Fibrin glues are often used to augment dural closures, although their actual efficacy is unknown[1]. Drawbacks limiting use are difficult preparation, clot strength, tissue adherence, and the concerns of immunogenicity and viral transmission.

Recently a new synthetic hydrogel, DuraSeal Dural Sealant, was shown to effectively seal dura in a canine cranial sealing model[2]. The sealant is polyethylene glycol (PEG) based, and transforms from a liquid to a solid within 2 seconds of spraying onto tissue, without detectable heat evolution. The resulting hydrogel is over 90% water, and contains a dilute blue dye to help visualization. The flexible hydrogel sealant is tissue adherent and strong enough to withstand intracranial pressures. After the dura has healed, hydrolyzable linkages within the PEG backbone allow the sealant to break down, absorb, and undergo renal clearance within 1-2 months. The current study evaluates DuraSeal absorption using MR and CT imaging following implantation in a canine craniotomy model.

Surgical Procedure

Two male beagles (mean weight 16.7 kg) were used in this study conducted at Brigham and Women's Hospital and at the Harvard Center for Minimally Invasive Surgery, Boston, MA in compliance with the Institutional Animal Care and Use Committee. Surgical procedures were conducted using routine sterile techniques.

Under general inhalant anesthesia, a curvilinear incision was made in the right frontoparietal region. The temporalis muscle was reflected laterally, three burr holes were created and a triangular shaped bone flap was raised with a Gigli saw. The dura was not incised in this study.

Following the confirmation of hemostasis, DuraSeal Dural Sealant (Confluent Surgical, Waltham, MA) was applied to the dural surface up to a thickness of 3 mm, approximately the same thickness as the bone flap (see Figure 1). The sealant contains a blue dye, which improves estimation of the resultant hydrogel thickness.

The bone flap was replaced over the DuraSeal and secured using 2-0 Vicryl sutures. The overlying muscle and skin were then sutured closed. Animals were returned to their cages for recovery, where they had free access to food and water.

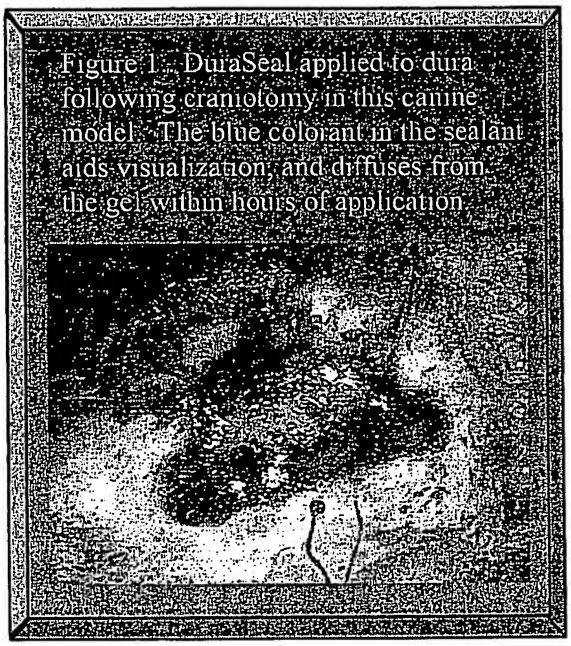


Figure 1. DuraSeal applied to dura following craniotomy in this canine model. The blue colorant in the sealant aids visualization, and diffuses from the gel within hours of application.

Imaging

Anesthesia

At each imaging time point, animals were sedated with a cocktail of xylazine, atropine, and ketamine, intubated, and maintained under anesthesia with Isoflurane (mean 2.5 hours) using an MRI compatible animal anesthesia machine (SurgiVet, Waukesha, WI). Animals were positioned in dorsal recumbancy, head first in both the CT and MRI scanners.

CT imaging protocol

CT imaging was performed on a Somatoma Plus 4 (Siemens AG, Erlangen, Germany). Images were acquired with a continuous spiral from C1 to the vertex (0 gantry tilt, standard algorithm with 2.5 mm slice thickness and spacing, table feed 3.75 mm per second, 3:1 pitch with HQ mode, 140 kV, 170 mA, 1 second rotation time, and 20 cm FOV). Images were reconstructed with a soft tissue/brain filter and window.

MRI imaging protocol

MR images were acquired on a 1.5T CV/i MRI scanner (General Electric Medical Systems, Milwaukee, WI) using a receive only 6" surface coil. T2-weighting, FLAIR (fluid attenuated inversion recovery), pre-contrast T1-weighted, and post-contrast T1-weighted images were acquired in the axial and coronal planes. In-plane resolution was 0.7 x 0.75mm^2 for FLAIR imaging and 0.7 x 0.7mm^2 for all other imaging. A dose of 2cc Gd DTPA (Magnevist, Berlex Labs, Wayne, NJ) was administered five minutes prior to post-contrast T1-weighted imaging. The complete protocol is shown in Table 1.

Images from only one animal are shown, so that the progression of DuraSeal absorption can be viewed in the same animal (Figure 2).

Table 1. MR Imaging Protocol

Sequence	T2 FSE	FLAIR	T1 SE pre	T1 SE post
Options	Fast	FC, VBW, Fast, IR	VBW	VBW
TE (ms)	85	120	9	9
TR (ms)	2000	9000	550	550
TI (ms)	N/A	2200	N/A	N/A
FA (degrees)	N/A	N/A	N/A	N/A
ETL	16	12	N/A	N/A
RBW (kHz)	15.6	15.63	15.6	15.6
FOV (cm)	18	18	18	18
ST (mm)	4/1	4/1	4/1	4/1
MATRIX	256 x 256	256 x 192	256 x 256	256 x 256
NEX	2	1	1	1
Frequency DIR	R/L	R/L	R/L	R/L
Num of slices	18	18	18	18
Acquisition time	3:24	4:12	2:28	2:28
Num of acquisitions	3	2	1	1
Slices per acquisition	7	14	22	22

FC: flow compensation, VBW: variable bandwidth, IR: inversion recovery, TE: echo time, TR: repetition time, FA: flip angle, ETL: echo train length, RBW: receiver band width, FOV: field of view, ST: slice thickness / spacing, NEX: number of excitations (signal averages).

Figure 2: Coronal images showing DuraSeal at 3 days, 2, 4, 6, 8, and 10 weeks (top to bottom) with T2-weighted, FLAIR, T1-weighted pre-contrast, T1-weighted post-contrast, and CT imaging (left to right).

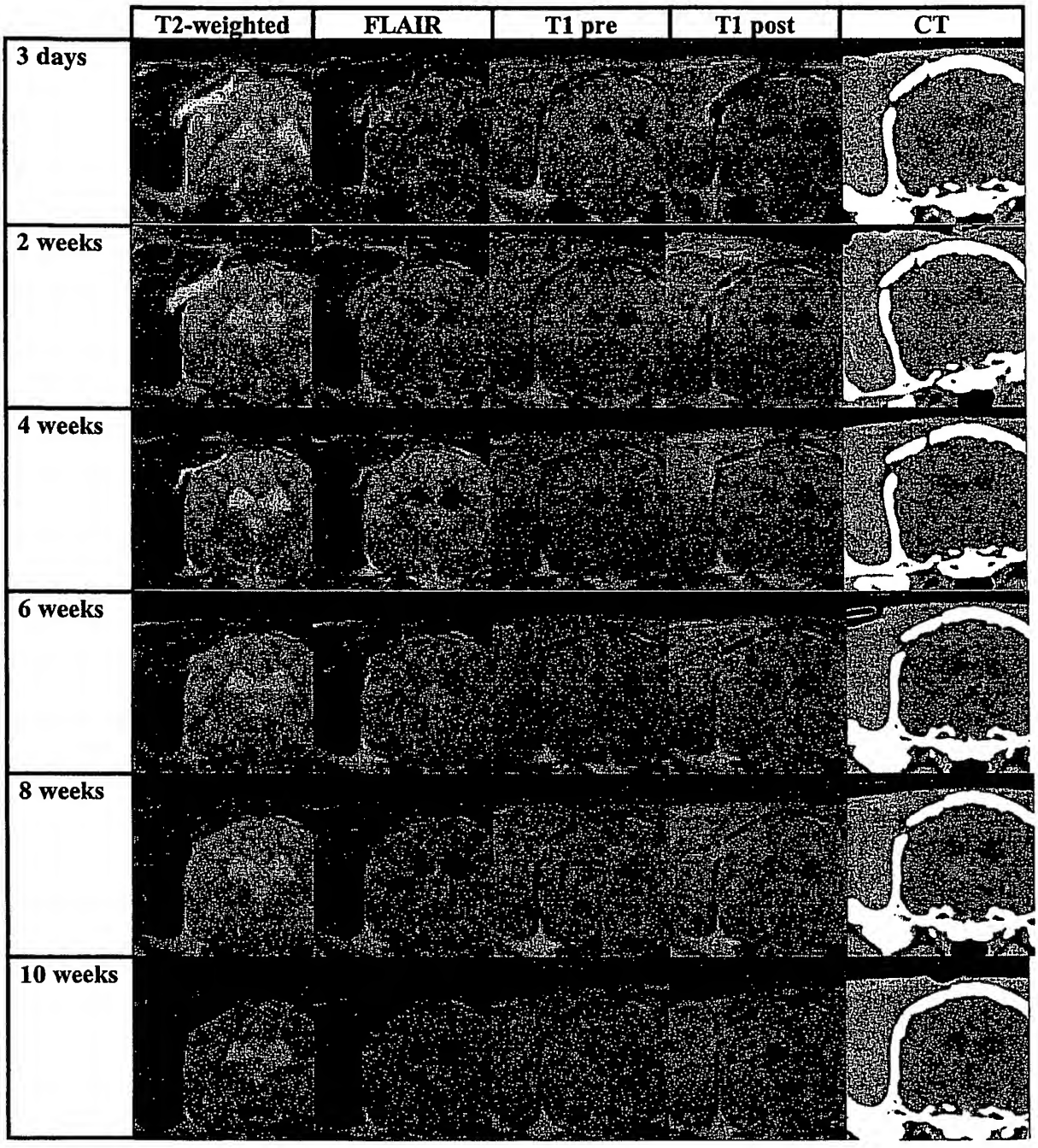


Image interpretation

The images can be analyzed with respect to the appearance of the skull, the cortex, the hydrogel, and hyperemia. The most helpful MR images demonstrating the presence of the hydrogel and characterizing the surrounding tissues appear to be the T1-weighted FLAIR images. The T1 post -contrast images are especially helpful in demonstrating the reactive enhancement surrounding the hydrogel. Similarly, the CT scan also demonstrates the enhancement pattern seen in the T1 post-contrast images, due to reactive tissue enhancement. Moreover, the CT demonstrates the presence of the hydrogel with the same sensitivity as the FLAIR images, although not as well as the T1-weighted images.

With the 3 day time point as a baseline, the temporal evolution of the hydrogel appearance can be described.

Week 1

The hydrogel is iso-intense to cortex in FLAIR images. Subtle streak-like hyperintensities possibly representing blood products are noted in the hydrogel in the T1-weighted images. Contrast enhancement is clearly demonstrated between the hydrogel and brain. Slight adjoining cortical surface enhancement is appreciated on the CT images. Slight cortical displacement secondary to hydrogel-related mass-effect is also demonstrated.

Week 2

MRI sequences show that the hydrogel appears to have further increased in thickness since the first imaging time point, and the surface adjoining the cortex appears smoother. The T1 post contrast images clearly demonstrate enhancement between the hydrogel and cortex and new enhancement between the hydrogel and calvarium. CT images also exhibit increased hydrogel volume with enhancement easily appreciated between the hydrogel and cortex. It is not easy to appreciate enhancement adjacent to the hyper-attenuating calvarium on the CT images, although the enhancement is more clearly shown on MR imaging. The hydrogel is most conspicuously seen on the T2-weighted and T1 post-contrast images.

Week 4

The greatest interval reduction in hydrogel thickness is noted in this scan set, with a large amount of interval absorption when compared with week 2. On FLAIR images, there is a new hyper-intense portion of the hydrogel next to the cortex, which is difficult to separate from the enhancing tissue shown on T1 post-contrast images. There is a significant change in the CT appearance of the bone flap with elevation of the lateral margin.

Week 6

CT and FLAIR images appear essentially normal, suggesting full absorption of the hydrogel. However, T2- and T1-weighted images demonstrate residual hydrogel persistence, and bed enhancement.

Week 8

The CT changes implying the presence of the hydrogel are no longer appreciable. The cortex has moved into its normal position. Beam hardening obscures fine juxta-calvarial detail. On T2-weighted images, there still is demonstration of extra-axial hydrogel.

Week 10

T1-weighted images demonstrate absorption of the hydrogel since week 8. This change is demonstrated with a greater confidence than afforded with the T2-weighted images. Residual contrast enhancement of the hydrogel bed is still shown at 10 weeks.

Discussion

The hydrogel is easily visualized with both CT and MRI from instillation through the four-week imaging interval, and becomes progressively more difficult to see from weeks six through ten. By ten weeks post implantation, it is very difficult to appreciate any residual hydrogel. The greatest confidence in visualizing the hydrogel is with the T2-weighted and T1 contrast-enhanced images.

In terms of the temporal changes demonstrated with the hydrogel, there are conspicuous

changes, which take place with the volume of hydrogel, in addition to changes, which take place with regards to the signal and enhancement characteristics of the hydrogel. There is demonstration of slight swelling of the hydrogel between day three and week two, simultaneous with an increase in superficial and deep adjoining circumferential enhancement; maximum hydrogel-adjoining enhancement is shown at week 2. The central portion of the hydrogel does not enhance, only the periphery of the hydrogel shows a shell-like rim of enhancement. The greatest change in volume takes place between weeks 2 and 4 with rapid resorption of hydrogel, simultaneous with a reduction in marginal enhancement intensity. There is a gradual ongoing reduction in the volume of hydrogel and the conspicuity of the hydrogel-adjoining enhancement, until the 10 week time point, when there is near total resorption with virtually no residual enhancement

Clinically, based on the appearance of the hydrogel, there may be a question of imaging-characterisics overlapping with either a persistent unilocular fluid collection such as a pseudomeningocele, or of greater concern, the imaging characteristic overlap with an infected surgical bed or infected fluid collection.

FLAIR imaging should be useful in differentiating the hydrogel from a pseudomeningocele. The hydrogel collection should appear hyperintense to CSF found in a pseudomeningocele, paralleling gray matter more closely in terms of signal characteristics than CSF.

Inflammatory collection, suggestive of infection, is best appreciated with T1-weighted imaging. The collection would be expected to have greater signal heterogeneity than that shown on the hydrogel-implant images. The symmetric and homogeneous circumferential marginal enhancement, which was limited in exuberance to the first two weeks, should be helpful in differentiating the adjoining hydrogel-implant from an infected surgical bed.

Similarly, with the uncomplicated single-sheet hydrogel implant there should be no enhancing tran-hydrogel bands, or central hydrogel enhancement. In the event that MRI and CT are unable to confidently exclude infection, then an indium labeled white-blood cell nuclear medicine study may be necessary.

Concerning the role of CT scanning in the characterization of the hydrogel collections, it is reassuring to see the similarity in the hydrogel volume changes and enhancement characteristics adjoining the hydrogel implants when comparing the CT to the MRI studies. Nevertheless, since questions regarding fluid collections and inflammation/infection may arise, if chronological growth of the hydrogel bed beyond two weeks is observed, or if there is complex central hydrogel enhancement or enhancement shown increasing in intensity beyond the two-week point, then MRI examination may be necessary to further characterize the bed. In the exceptionally rare instances where further investigation may be warranted beyond MR imaging, indium-labeled white-blood cell nuclear medicine scanning should be considered.

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CONFERENCE REPORT

The use of Human Fibrin Glue in the surgical operations

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Abstract. Human Fibrin Glue (HFG) is made of two components contained in separate vials: a freeze dried concentrate of clotting proteins, mainly fibrinogen, Factor XIII and fibronectin (the sealant) and freeze dried thrombin (the catalyst). The first component is reconstituted with an aprotinin solution that inhibits tissue fibrinolysis. The second component (thrombin), available in 500 I.U. concentration, is dissolved with calcium chloride. It is so a set of substances involved in the hemostatic process and in the wound healing, conferring to the product the following important properties: hemostatic and sealing action, through the strengthening of the last step of the physiological coagulation; biostimulation, which favors the formation of new tissue matrix. The indications for the use of human fibrin sealant are numerous and present in all the surgical branches. A randomized controlled trial of 50 patients undergoing hernia repair according to Lichtenstein's technique under local anesthesia was performed. Patients had concurrent coagulopathies as a consequence of liver disease or long-term treatment with anticoagulants for ischemic heart disease or cardiac rhythm disturbances. Coagulopathies were defined according to the following criteria: prothrombin time <10.5 seconds, activated partial thromboplastin time < 21 seconds, and fibrinogen <230 mg/dL. Patients were randomized in a 1:1 ratio with (group A) or without (control group B) use of human fibrin glue. Postoperative hemorrhagic complications were significantly reduced in group A (4%) compared with group B (24%). This study showed that human fibrin glue is effective in preventing local hemorrhagic complications after inguinal hernia repair in patients with concurrent coagulation disorders.

Key words: Human fibrin glue, hemostasis, hernia repair, surgery

Introduction

The surgical suture, both when realized with the traditional threads and with the most modern mechanical staplers, represents the "classic" method for wound repair. All surgeons, however, sometimes have to deal with wounds hard to recover because, due to stretching and ischemic events of variable extent, complications such as hematomas, granulomata, dehiscences and fistulae may occur, impairing the tissue healing.

Therefore, the ideal solution would be to have wound healing with no need of sutures, capable to sustain a certain mechanical stretching and with optimal conditions for a rapid recovery without leaving foreign substances in the wound area. On the other hand this concept, even in an empiric way, was already known by the ancient doctors of Great Greece who used vegetal resins for wound healing purposes.

Fibrin's properties, interactions, mechanical strength and resistance to fibrinolysis have been thoroughly studied and described in many publications. Fibrin sealant was developed in 1972 by Matras et al., who successfully used a fibrinogen cryoprecipitate in peripheral nerve anastomoses on animal models. The development of a special cryoprecipitation process enabled the production of a high concentration fibrinogen solution with a high Factor XIII content. Moreover, the introduction of aprotinin, a natural antiproteasic substance, allowed to solve other problems, such as fibrinolysis inhibition and early fibrin degradation.

Characteristics, properties and modalities of application

Fibrin sealant is made of two components contained in separate vials: a freeze dried concentrate of clotting proteins, mainly fibrinogen, Factor XIII and fibronectin (the sealant) and freeze dried thrombin (the catalyst). The first component is reconstituted with an aprotinin solution that inhibits tissue fibrinolysis. The second component (thrombin), available in 500 I.U. concentration, is dissolved with calcium chloride.

It is so a set of substances involved in the hemostatic process and in the wound healing, conferring to the product the following important properties:

- hemostatic and sealing action, through the strengthening of the last step of the physiological coagulation;
- biostimulation, which favors the formation of new tissue matrix.

Let's examine the main components of Tissucol and its most important mechanisms of action by evaluating the effects on hemostasis, adhesion and tissue healing.

Fibrinogen. It is a high molecular weight protein, precursor of fibrin, which represents the basic element of the clot. The transformation of fibrinogen into stable fibrin occurs by means of thrombin and factor XIII, which in turn are activated by thrombin. Fibrin has a strong affinity with the factors derived from plasma or of cellular origin and with collagen. The fibrin clot that forms in a wound seems to act as a guide to the healing process, attracting the fibroblasts and promoting the formation of granulation tissue.

Factor XIII. It is a transglutaminase, carrying out some important functions:

- it catalyzes the formation of intermolecular bridges between a lysin and a glutaminic residue of fibrin molecular chains;
- it stabilizes with the same mechanism the link between fibronectin and fibrin and between fibronectin and collagen, thus increasing the molecular stability of the three-dimensional network of the clot;
- it links alpha2 antiplasmin to the clot, so slowing down the action of plasmin and consequently regulating the fibrinolysis.

Fibronectin. It is a high molecular weight glycoprotein, which:

- interacts with fibrinogen and preferentially with polymerized fibrin, forming covalent links catalyzed by Factor XIII, which lead to the concentration of both molecules: fibronectin is then an integral part for adhesion and migration of fibroblasts to the wound area;
- it gets linked to collagen with molecular affinity interactions: this link favors the interaction of collagen with other reparation structures, favoring the regeneration of a new matrix;
- it interacts with the cells, and this adhesion to fibronectin seems to be very important for various types of cells;
- it is also a substrate for Factor XIII.

The main characteristics of fibronectin consist of participating in the formation of the mixed fibrin clot, of increasing the adhesive property between cells and substrate and between one cell and another, and of favoring their growth and migration.

Aprotinin. It is a polypeptide that blocks plasmin and other serin-proteases. Through the addition of aprotinin, it has shown to be the best inhibitor of fibrinolysis which can be delayed, resulting in a significant increase of the healing processes, enhancing the formation of connective tissue. Thus, the survival of fibrin sealant can be controlled through suitable dosages of aprotinin, after having evaluated the fibrinolytic activity of the relevant area.

Thrombin. It is a serum protease with important functions: the transformation of fibrinogen into fibrin, activation of Factor XIII and stabilization of the network formed by fibrin and other proteins are the main ones.

Calcium chloride. Ca++ ions are indispensable in various coagulation steps, for example in the transformation of prothrombin into thrombin and in the activation of Factor XIII; the polymerization processes are accelerated by the presence of Ca++ ions.

In order to make the preparation and application of Tissucol easier and more effective, in the various indications, special devices and methods have been developed (application needle, Tissuspray, DuoFlo, Duplo-Tip and catheters), enabling to use the product in different areas – constricted, large or difficult to access.

Indications

The properties of the main components, the possibility to add variable quantities of antifibrinolytic substances and to influence the consolidation time, the availability of special application devices, make the product extremely easy to handle and with practically limitless application fields in surgery. Its therapeutical significance and tolerability have been demonstrated at the international level in many fields of general and specialistic surgery, both as the sole suture element and as adjuvant of other means of surgical synthesis and hemostasis. In the light of its peculiar characteristics, many authors have specifically evaluated this product in patients with coagulation disorders, with absolutely specific problems and risks in case of surgical treatments.

The indications for the use of human fibrin sealant, also thanks to evident practical and economic advantages of the product, are numerous and present in all the surgical branches. The most significant are the following ones.

- *Abdominal and general surgery*

The adhesive, sealing and hemostatic properties of Tissucol are particularly useful in this kind of surgery. The hemostatic property, in particular, is helpful in parenchymal surgery, mostly in liver, pancreas and spleen surgery, above all in case of partial resections and when it is necessary to favor the hemostasis of large injured areas, with massive bleeding. The considerable reduction of blood loss, correlated to seeping hemorrhages, diminishes blood transfusions or even makes them unnecessary. The use of the preparation also allows to avoid additional traumas to the residual parenchyma, reducing the suture stitches that strongly harm such friable tissues (1-5). In pancreatic surgery, fibrin sealant has been used also to treat the pancreatic stump with no pancreo-digestive anastomosis (6).

The adhesive action of Tissucol is very useful in intestinal surgery, where it is necessary to carry out a complete and tight suture of the walls, which is not always possible. The use of Tissucol allows to obtain a complete and integral reparation of the tunicae, optimally resuming the continuity and tightness of the intestinal tract: many authors have carried out experimental and clinical researches which have demonstrated a reduction in the rate of dehiscences, both in the manual and mechanical anastomoses (7). Human fibrin glue has also been used to treat fistulous complications of digestive anastomoses in a conservative way, without having to re-operate (8). The use of Tissucol, for the same reasons, has shown to be particularly useful for the non-surgical treatment of anal and perianal fistulae (9).

Moreover, excellent results have been described for the prevention of axillary lymphorrhea after breast surgery (10), as well as for the prevention of complications in prosthesic surgery of hernias, both tension-free and laparoscopic (11).

- *Thoracic and cardiovascular surgery (12-15)*

These types of specialistic surgery benefit by the adhesive, hemostatic and sealing properties of Tissucol in many indications.

In cardiosurgery, the hemostatic and adhesive properties, are particularly crucial especially in coronary surgery, in case of valve plasty and in congenital cardiac pathologies, both with or without the use of prostheses. Furthermore, the preparation has enabled to obtain extraordinary therapeutical results in the surgery of dissecting aneurysms. Tissucol has shown to be a valuable tool to solve contingent situations and technical problems, sometimes life-threatening.

In peripheral vascular surgery, the sealing property is particularly useful. This type of surgery could be in practice compared to microsurgery, where the possibility to avoid sutures is certainly advantageous. Moreover, a rapid sealing allows to obtain a stable consolidation within short time, certainly sooner than with the traditional sutures.

In thoracic surgery, the indications to the use are mainly bronchial and parenchymal sutures, where the control of air leak through the sealing properties results in a post-surgical rapid and safe course. In pulmonary surgery, if a complete aerostasis is not achieved, the time of hospitalization increases because of the necessity to perform thoracic drainages.

- *Urological surgery (16)*

Also in this field, fibrin sealing can represent a valid technical tool in many procedures. The complete

compatibility with the tissues, as well as the hemostatic and sealing effects for anastomoses, confer to this product some irreplaceable peculiarities, allowing to realize tissue synthesis in the best possible way. In urological surgery, in fact, it is necessary that sutures, being in direct contact with urines, are carried out in such a manner to avoid the possible occurrence of an urinose fistula. Just the adhesive property of Tissucol for any tissue allows to consolidate within a very short time the traditional sutures, making them more airtight. It is also extremely important the possibility to obtain a safe and perfect hemostasis in the presence of parenchymal sections, as in the conservative kidney surgery, or of large injured areas with seeping bleedings, as for example in the pelvic excavation after total cystectomy.

- *Plastic surgery (17-20)*

In this field there are many indications mostly related to the adhesive property. The take of the free flaps of transplanted skin is conditioned by two factors: the adhesion of the flap that has to be as much complete as possible and immediate, and the absence of hematomas that cause the failure of the take. Fibrin sealant, due to its sealing property, together with the hemostatic property, thus represents an ideal tool. Moreover, it can allow the biological cover in large injured areas, so representing a biologically favorable bed for skin transplantation. At last, the application of the product is particularly suitable for suturing delicate areas such as face or eyelids, or to be used in the skin cover of fingers.

Personal experience (21)

Our purpose was to establish the efficacy of human fibrin glue in preventing coagulative complications after inguinal hernia repair in patients with coagulation disorders.

A randomized controlled trial of 50 patients undergoing hernia repair according to Lichtenstein's technique under local anesthesia was performed. Patients had concurrent coagulopathies as a consequence of liver disease or long-term treatment with anticoagulants for ischemic heart disease or cardiac rhythm disturbances. Coagulopathies were defined according to the following criteria: prothrombin time <10.5 seconds, activated partial thromboplastin time <21 seconds, and fibrinogen <230 mg/dL. Patients were randomized in a 1:1 ratio with (group A) or without (control group B) use of human fibrin glue.

Postoperative hemorrhagic complications were significantly reduced in group A (4%) compared with group B (24%).

This study showed that human fibrin glue is effective in preventing local hemorrhagic complications after inguinal hernia repair in patients with concurrent coagulation disorders. This implies that the use of human fibrin glue reduces the costs of prolonged hospitalization related to such complications.

Conclusions

The important role of the biological sealant in surgery is highlighted by the long experience acquired on international level: in fact, so far more than 3,000 scientific papers have been published and millions of patients have been treated. The literature has always confirmed the effectiveness of the product and has also demonstrated the excellent local tolerability and the complete absence of undesirable effects and contraindications.

The authors often report also the favorable cost-benefit ratio, mainly due to the reduction of the hospitalization time, thanks to the rapid wound healing, to the early drainage removal and to the reduction of complications such as hematomas, sepsis, dehiscences and formation of fistulae (21, 22).

In conclusion, it is possible to affirm that Tissucol has to be considered a product with an excellent efficacy-safety ratio, as a result of more than 30 years of experience and experimentation. We can certainly declare that thanks to its properties, it has allowed to obtain considerable advantages, for example the possibility to improve the surgical procedures and in some cases to realize new techniques, hard to achieve until then.

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Feasibility Study of NeoMend, a Percutaneous Arterial Closure Device That Uses a Nonthrombogenic Bioadhesive

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OBJECTIVE. The aim of this prospective single-center phase I feasibility study was to investigate the safety and efficacy of a novel vascular sealing device, the NeoMend Arterial Closure Device, that uses a bioadhesive after percutaneous endovascular procedures.

SUBJECTS AND METHODS. In 26 consecutive patients, the sealing device was deployed at the femoral artery access site immediately after a catheterization procedure using a 6-French (1.91-mm) sheath. Patients were followed up at 24 hr with Doppler sonography of the treated femoral artery puncture site, and at 1 week and 1 month by a telephone interview.

RESULTS. Successful hemostasis was achieved with the NeoMend Arterial Closure Device in 21 (88%) of 24 patients. One major complication required surgery: formation of puncture site hematoma and pseudoaneurysm 3 days after the intervention after successful primary hemostasis. Two device failures required crossover to manual compression, which was done without further complications. The mean time to hemostasis was 7.0 ± 4.5 min. Mean time to ambulation was 6.0 hr. At follow-up, the patients did not report any puncture-site-related complaints. Doppler sonography of the puncture sites revealed three insignificant hematomas of less than 20 mL and patent common femoral vessels without stenoses.

CONCLUSION. The NeoMend Arterial Closure Device appears to achieve rapid hemostasis with the potential of early ambulation after arterial punctures with a 6-French sheath. The device is an alternative in situations in which suture- or collagen-mediated devices show high complication rates.

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Angiographic procedures involving arterial puncture carry a risk of access site complications, which are estimated to occur in 1–5% of procedures [1]. The risk of significant vascular injury may be as great as 14% in certain interventional procedures, particularly those requiring thrombolysis or prolonged anticoagulation [2]. Therefore, new methods to assist with hemostasis at the time of sheath removal are of considerable interest [3]. This interest is further fueled by an increasing emphasis on outpatient catheterization and a desire for early mobilization [4–7].

Some currently marketed devices use a collagen plug that has resulted in a decreased time to ambulation in several studies [8–10]. However, other studies [11] could not show the superiority of collagen sealing compared with manual compression. Some investigators found a higher complication rate, including acute femoral artery occlusion, with collagen than with manual compression [12]. In addition, with such devices, repeated access at the same site is impaired until complete resorption of the intravascular anchor of the collagen plug has been achieved.

A different approach involves the development of percutaneous vascular closure devices that deliver needles and sutures through the arterial wall around the access site. After reports of initial success [13], studies of large populations have shown a significantly higher complication rate after diagnostic procedures with such devices than with manual compression [14]. A more recently discussed limitation is the greater incidence of local infection that has been linked to the introduction of a foreign body [15].

None of these devices is a completely satisfactory solution to maximizing patient safety and comfort or to a shortened hospital stay [16]. The purpose of our study was to test the feasibility of a novel arterial closure device (NeoMend Arterial Closure Device; NeoMend, Sunnyvale, CA) that applies a rapidly and completely resorbable bioadhesive in the puncture canal to allow early mobilization after angiography. The

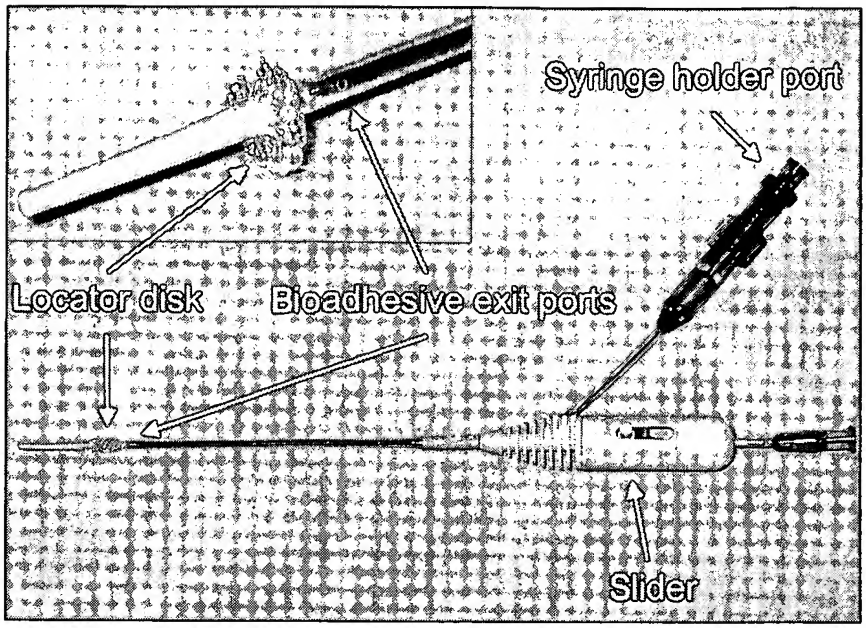

Fig. 1.—Photograph shows NeoMend bioadhesive applicator device (NeoMend, Sunnyvale, CA).

potential advantages of this closure technique are the possibility of immediate repuncturing on the same site and the lack of excessive scar formation because of the fast resorption and biocompatibility of the adhesive, thus facilitating eventual subsequent surgery and reducing the risk of puncture site infection. To show the biocompatibility of the bioadhesive, a program of in vitro and in vivo biocompatibility testing was completed in accordance with International Standards Organization 10993-1, which regulates the selection of biocompatibility tests for a medical device [17]. The bioadhesive has been categorized as a permanent exposure implant (> 30 days, blood contact).

Subjects and Methods

The investigation was designed as a prospective single-center feasibility study in consecutive patients undergoing diagnostic or interventional angiography. The device studied has not yet been approved for clinical use; the investigational protocol and the informed consent forms were endorsed by the institutional ethics committee. All patients gave informed consent to the use of the device and were made aware of potential associated risks in accordance with the Declaration of Helsinki. Specific risks the participants were advised of included insufficient closure of the arterial puncture, immediate or delayed bleeding, formation of a hematoma or pseudoaneurysm that would eventually necessitate surgery, peripheral nerve injury, occlusion of the femoral artery or distal embolization with acute limb ischemia, allergic reaction to the bioadhesive, puncture tract infection, and abscess formation. In accordance with the guidelines of the institutional ethics committee, liability insurance was provided for every participant covering all potential device-related complications or adverse effects.

Enrollment

Between July 2001 and January 2002, 26 consecutive patients were included in the study. The mean patient age was 66.6 ± 10.8 years. Exclusion criteria were age younger than 18 years, pregnancy, ipsilateral prior femoral access within 30 days, history of prior femoral closure with another device, bleeding disorders (including thrombocytopenia, activated clotting time > 300 sec), persistent hypertension (systolic blood pressure > 180 mm Hg or diastolic blood pressure > 110 mm Hg), planned prolonged heparin or warfarin therapy, significant anemia (hematocrit < 30%), preexisting hematoma, or known sensitivity to polyethylene glycol.

Intervention and Medication

In the morning before the intervention, blood samples were drawn and the activated partial thromboplastin time (aPTT) was measured. All patients underwent retrograde common femoral artery single-wall puncture under local anesthesia using the Seldinger technique with a 0.035-inch guidewire and the placement of an introducer sheath with an inner diameter of 6 French (2.0 mm) and an outer diameter of 7.2 French (2.4 mm).

Eleven of 26 patients did not receive anticoagulants during the intervention. Fifteen of 26 patients received 5000 U of unfractionated heparin sodium intraarterially and a daily dose of 100 mg of aspirin and 75 mg of clopidogrel (Plavix; Bristol-Meyers Squibb, Vienna, Austria) starting immediately after the intervention for a minimum of 6 weeks.

NeoMend Device

The Neomend Arterial Closure Device consists of two components: the bioadhesive and the delivery device. The arterial closure device is designed to seal arterial punctures from up to 7-French sheaths with an outer diameter of 8.4 French (2.8 mm). The device is depicted in Figure 1. Its total length is 176 mm, with an intravascular part 100 mm long and an outer diameter of 8 French (2.4 mm).

The bioadhesive is a two-part mixture consisting of a polymer component (polyethylene glycol) and a protein component (human serum albumin). Mixing of the two components occurs during injection, and cross-linking of the bioadhesive occurs in situ to establish hemostasis at the femoral artery puncture site.

The distal end of the delivery device (Fig. 1) incorporates a nylon open-weave mesh locator disk that can be deployed by moving the slider on the device handle. The distal tip of the catheter shaft is radiopaque, and a radiopaque marker is proximal to the locator disk. Four to six millimeters proximal to the deployed locator disk are four exit holes for the bioadhesive. With the locator disk deployed and retracted to the inside of the vessel wall, the bioadhesive is delivered to the exterior surface of the artery and the puncture canal to establish hemostasis.

The syringe holder is a dual-chamber cartridge with cavities and Luer-Lock connectors for each syringe containing the bioadhesive constituents. The plunger of the syringe holder allows simultaneous depression of the plunger on each syringe.

Delivery of Bioadhesive and Hemostasis

The delivery catheter was placed over a compatible guidewire in the arterial puncture site. The catheter tip was advanced into the artery. The locator disk on the catheter was deployed, and the catheter shaft was withdrawn until the disk made contact with the inside wall of the artery. The dual-chamber syringe holder (with bioadhesive syringes loaded) was connected to the injection port on the delivery catheter. The plunger on the syringe holder was advanced, injecting the bioadhesive through the catheter to the area of the arterial puncture. The locator disk was collapsed, and the catheter and guidewire were withdrawn from the puncture within 30 sec (Fig. 2).

Puncture Site Management

After the application of the bioadhesive, manual compression was initiated for 5 min to allow the material to polymerize. The puncture site was assessed by complete removal of manual pressure every 5 min until complete hemostasis was confirmed. The total time necessary to achieve complete hemostasis was recorded. A compression bandage with a single transverse adhesive patch was applied.

TABLE 1 Results of Using Percutaneous Arterial Closure Device in 26 Study Patients Shown in Chronologic Order

Patient		aPTT[a] (sec)	Heparin (U)	No. of Exchanges[b]	Catheter Time (min)	Success[c]	Time to		Major Complications	Follow-Up		
No.	Age (yr)						Hemostasis (min)	Ambulation (hr)		24 Hr	1 Week	1 Month
1	74	36.6	5000	1	55	Yes	10	8	Yes	Normal	Postoperative[d]	Postoperative
2	61	43.8	0	1	70	Yes	10	8	No	Normal	Normal	Normal
3	61	45.8	0	1	60	Yes	10	4	No	Normal	Normal	Normal
4	68	38.5	5000	2	50	Yes	5	6	No	Normal[e]	Normal	Normal
5	71	41.2	0	1	20	Yes	5	6	No	Normal	Normal	Normal
6	60	34.6	0	1	55	Yes	5	6	No	Normal	Normal	Normal
7	47	37.2	5000	3	35	Yes	5	4	No	Normal	Normal	Normal
8	60	43.9	5000	3	40	No[f]	25	24	No	Normal[g]	Normal[g]	Normal[g]
9	79	36.4	0	1	20	Yes	5	6	No	Normal[e]	Normal	Normal
10	62	34.5	0	1	15	Yes	5	4	No	Normal	Normal	Normal
11	47	40.5	0	1	20	No[h]	25	24	No	Normal[g]	Normal[g]	Normal[g]
12	76	36.0	5000	2	65	Yes	5	6	No	Normal	Normal	Normal
13	71	34.0	5000	2	50	Yes	5	6	No	Normal	Normal	Normal
14	64	40.7	5000	2	40	Yes	10	6	No	Normal[i]	Normal	Normal
15	82	29.6	5000	2	110	Yes	5	6	No	Normal	Normal	Normal
16	82	37.7	0	1	20	Yes	10	6	No	Normal	Normal	Normal
17	63	35.2	0	1	65	Yes	5	6	No	Normal	Normal	Normal
18	76	40.1	5000	3	60	Yes	5	6	No	Normal	Normal	Normal
19	76	36.8	5000	2	100	Yes	5	6	No	Normal	Normal	Normal
20	67	36.2	5000	2	80	Yes	5	6	No	Normal	Normal	Normal
21	81	36.2	5000	2	40	Yes	5	6	No	Normal	Normal	Normal
22	43	30.9	5000	2	55	Yes	5	6	No	Normal	Normal	Normal
23	64	37.3	0	1	120	Yes	5	6	No	Normal	Normal	Normal
24	49	32.1	5000	2	35	Yes	5	6	No	Normal	Normal	Normal
25	72	36.8	5000	3	30	Yes	5	6	No	Normal	Normal	Normal
26	65	31.0	0	1	90	Yes	5	6	No	Normal	Normal	Normal

[a] Activated partial thromboplastin time.
[b] Sheath exchanges required during intervention.
[c] Primary technical success.
[d] Surgery because of pseudoaneurysm.
[e] Insignificant hematoma, 2 cm.
[f] Device failure.
[g] After manual compression.
[h] Conversion to manual compression.
[i] Insignificant hematoma, 1.7 cm.

Follow-Up Protocol

Two hours after the closure, the puncture site was inspected clinically. If no hematoma was palpable, the patient was encouraged to stand. After another inspection of the puncture site, the patient was transferred to the ward. The time to ambulation was measured from the completion of catheterization until the patient was able to walk three to five steps independently without any complications.

Twenty-four hours after the closure, color-coded duplex sonography of the puncture site was performed. Any stenosis, pseudoaneurysm, or hematoma was noted and quantified. Subjective patient complaints, such as local tenderness or pain, and clinical signs, such as swelling or discoloration of the skin, were assessed.

After 1 week and after 1 month, telephone interviews were undertaken in which the patients were questioned regarding local complaints about the puncture site or signs of limb ischemia. Patients with complaints were reassessed with a clinical examination, sonography, and further investigations as needed. All parameters were recorded on specific case report forms.

Study Definitions

The primary safety end point was the total number of major complications at 1 month after the intervention, and the primary procedural success end point was the number of patients (on an intent-to-treat basis) in whom hemostasis could be achieved within 10 min of manual compression after successful application of the bioadhesive. Major complications included hemorrhage, requiring surgery or transfusion; formation of a pseudoaneurysm, requiring sonographically guided compression or surgery; nerve injury; and infection of the puncture site, requiring antibiotic medication or surgery. "Adjunctive compression" was defined as any standard arterial compression beyond 10 min after application of the bioadhesive. "Failure to deploy the adhesive" referred to the inability to correctly position the device in the artery and was considered device failure. Non-adjunctive arterial compression due to ongoing hemorrhage after deploying the adhesive was termed "crossover to manual compression."

Results

Closure procedures were attempted in all 26 patients. In 25 of 26 patients, the device and the bioadhesive were deployed successfully. Clotting values were obtained 1–3 hr before the intervention. In 15 patients who did not receive anticoagulants, mean (±SD) activated partial thromboplastin time (aPTT) was 38.7 ± 4.0 sec. In 11 patients who underwent vascular interventions, mean aPTT was 36.3 ± 3.9 sec before the administration of 5000 U of unfractionated heparin sodium. The average catheter time of the interventions was 54 ± 28 min. Between one and three sheath exchanges (mean, 1.7 exchanges) were performed. Table 1 shows the details for all 26 patients.

Primary End Points

Four days after an initially successful closure procedure, one patient who underwent renal artery stenting developed inguinal hematoma and pseudoaneurysm, which required surgery. Four days postoperatively, the patient was discharged without any further complaints. Thus, the rate of major complications at 30 days was 4.2%.

For all patients, the mean time to hemostasis was 7.0 ± 4.5 min. For those 24 patients with initially successful device deployment, time to hemostasis was 6.1 ± 2.1 min.

Secondary End Points

Procedural success in obtaining permanent hemostasis was achieved in 23 of 26 patients. In addition to the one patient who required surgery, technical device failures occurred in two patients.

In one patient who had undergone previous bypass surgery, the locator disk disconnected from the applicator during the retraction, and the additional resistance of the disk against the arterial wall was hardly felt because of excessive scar formation around the puncture site, which made it difficult to retract the device at all. In this patient, the sheath was reintroduced and the disk was easily retrieved with a snare, followed by manual compression that was performed without complications. This device separation could not be repeated intentionally when the remaining devices of this lot were tested. In one patient, hemostasis was not achieved after deployment of the bioadhesive, and another 25 min of manual compression were required. This patient was not permitted to be mobile before 24 hr for safety reasons.

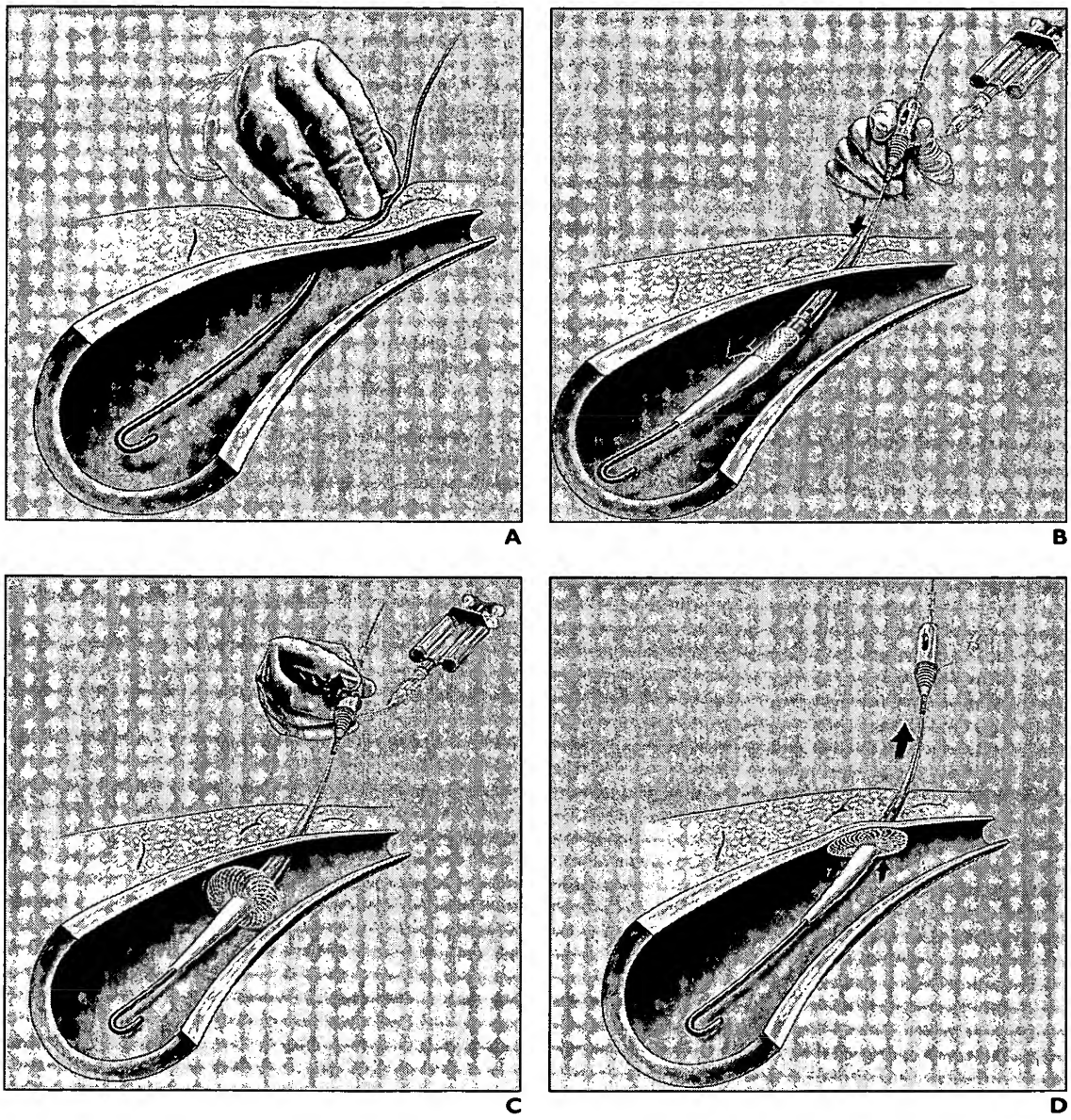

Fig. 2.—Schematic diagrams of NeoMend Arterial Closure Device (NeoMend, Sunnyvale, CA).
A, After completion of intervention, guidewire is left in puncture canal.
B, Device is inserted into femoral artery over guidewire.
C, Locator disk is deployed.
D, Device is pulled back until resistance from locator disk on arterial wall is felt.
Fig. 2 continues on next page.

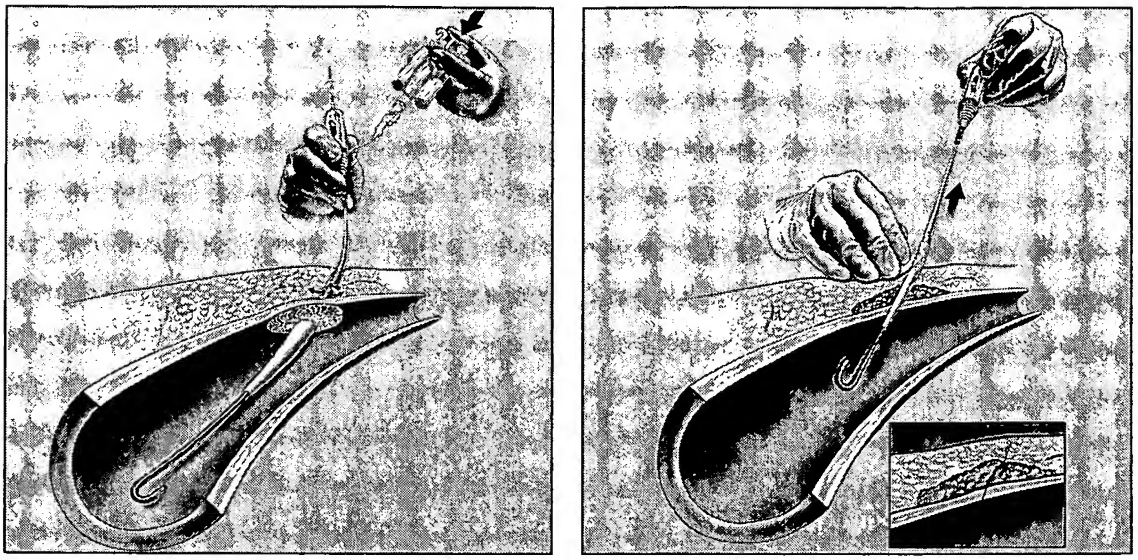

Fig. 2.—(continued) Schematic diagrams of NeoMend Arterial Closure Device (NeoMend, Sunnyvale, CA).
E, Bioadhesive is delivered.
F, After locator disk is collapsed, device and wire are withdrawn from artery under manual compression.

We noted a 12% (3/26 patients) rate of nonpalpable, sonographically detected, clinically insignificant hematomas smaller than 20 mL (<3.4 cm in diameter). The bioadhesive itself was visible on sonography only immediately after the application as a hypoechoic mass. At the follow-up investigation, the bioadhesive was isoechoic to the surrounding tissue. The results of Doppler waveform analysis did not reveal any sign of luminal abnormality in the punctured arteries. No arteriovenous fistulas or false aneurysms were detected. Clinical examinations and follow-up investigations did not show any signs of ischemia attributable to the bioadhesive closure. All femoral access sites healed without signs of inflammation or infection. No nerve injuries were detected.

Discussion

The results of our study show that bioadhesive-mediated percutaneous closure of arterial puncture sites in the groin is feasible. The rate of procedural success was 88% (23/26 patients). One patient had to undergo surgical repair, which put the rate of major complications at one (3.8%) in 26. This patient was the first patient in the series. Although all three operators who performed the closures underwent thorough instruction and a 1-hr training session on a model device before applying the bioadhesive in the clinical setting, a learning curve existed, as has been described for other hemostatic devices [18]. We expect that the frequency of complications will decrease with operator practice. In our experience, it is important to avoid exerting too much pressure on the puncture site after applying the bioadhesive and removing the device, in order not to distribute the material away from the puncture canal before polymerization occurs. Rather, pressure should just be sufficient to prevent extravasation of blood and to keep the bioadhesive in place. When polymerization occurs after approximately 40–60 sec, the pressure can be further decreased. That the major complication, the two device failures, and two of three minor hematomas occurred in the first half of the series can, at least in part, be attributed to a learning curve, especially concerning the technique of compression during and immediately after device removal.

In one patient, a device failure could be observed when the tip of the device with the locator disk separated from the device during retraction; this patient had extensive scar formation in and around the puncture canal. The locator disk was still on the guidewire and could easily be removed by placing a snare over the wire proximally to the locator disk and then removing the snare, the sheath, and the disk. However, such a device separation occurred only once in the series. The disk could not be pulled off the device by hand in the remaining devices of the same charge, so the problem was thought to derive from a single faulty device and a high resistance when the device was retracted. The manufacturer was informed of this problem, and additional testing of the connection between the device and the disk was incorporated into the quality control process.

The mean time to hemostasis in this study was 6.0 ± 2.1 min, which compares favorably with 9.6 min with collagen plugs and with 23.6–33.5 min with manual compression [19, 20]. This time is similar to the time required with suture-mediated devices, in which times to hemostasis of 5.3 ± 3.8 min have been reported [21].

Sealing the arterial puncture site immediately after diagnostic or interventional procedures improves patient comfort and may also reduce the length of hospitalization and the total procedural costs. Various sealing devices using different principles of operation have been investigated. Compared with manual compression, earlier hemostasis [22] and reduced patient discomfort [23] have resulted. However, to date none of the sealing devices has been shown to shorten the hospital stay, to decrease procedural costs, or to reduce major local complications when compared with manual compression [24]. On the other hand, some more recent studies with large collectives have reported significantly greater complication rates with sealing devices [12, 14, 15].

The approach described in this study has the potential to overcome some of the problems associated with other sealing devices. The device

is easy and straightforward to use with techniques familiar to the interventional radiologist. That the components of the bioadhesive are human serum albumin and synthetic polyethylene glycol avoids the potential hazards of bovine products and minimizes the risks of allergic sensitization or other immunologic responses. A readily resorbed polymer provides less potential for excessive scar formation, which can impede subsequent interventions or surgery at the puncture site and which are known to occur with devices that use a collagen plug for sealing. In addition, some of the collagen-plug devices place a resorbable anchor in the artery lumen, which makes a reintervention at the same site impossible until the anchor is completely resorbed [25]. Further, the NeoMend Arterial Closure Device does not depend on a largely intact and uncalcified vessel wall of the common femoral artery, which is often not available in diabetic or aged patients, as opposed to suture-mediated devices, which show significantly more technical failures in such patients [14, 26].

The major goal of this first feasibility study was to assess the safety and efficacy of a new approach to vascular sealing. As a result, the measured times to hemostasis and ambulation may have been artificially prolonged, particularly in patients undergoing diagnostic procedures. As noted previously, the compression times were evaluated at 5-min intervals, with most patients showing complete hemostasis after the first 5 min. Shorter assessment intervals might have revealed that the actual time to hemostasis was even shorter than that seen in this study. Similarly, no emphasis was placed on patients becoming ambulatory immediately after the procedure. Rather, patients were mobilized after a 6-hr safety interval, although they were allowed to stand at the bedside 2 hr after the intervention.

Another concern was the risk of inadvertent intraarterial administration of the bioadhesive. With other devices, insertion of hemostatic agents or thrombogenic device parts into the artery could lead to extensive thrombosis and femoral artery occlusion with resulting limb ischemia, infarction, and potential loss of the limb [25]. Previous preclinical experiments with the bioadhesive used in this device have shown that its ability for polymerization is confined to a narrow gap of high pH and high local concentrations of the polymer components. Intravascular application would lead to immediate dilution and a decrease in pH, thereby preventing embolus formation. Intraarterial injection of the whole dose in animal experiments did not lead to formation of any arterial emboli (Milo C, NeoMend; unpublished data). In our study, no evidence of device-related embolization or ischemia was seen.

In conclusion, the NeoMend Arterial Closure Device provides a feasible, effective, and safe means of hemostasis after arterial catheterization procedures. The device provides earlier hemostasis than manual compression and has the potential for earlier patient mobilization after interventional procedures, even in patients who received anticoagulation. This device may prove especially useful in interventional settings when access site complications are frequent or after diagnostic procedures when early mobilization can lead to a significant reduction in costs. These two applications are currently being confirmed in ongoing multicenter trials.

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ORIGINAL ARTICLE

Autologous Collagen Dispersion (Autologen) as a Dermal Filler

Clinical Observations and Histologic Findings

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Objective: To assess the histologic behavior and clinical efficacy of autologous collagen dispersion (Autologen) in augmenting human dermis.

Subjects: Adult patients of the Facial Plastic Surgery Clinic at The New York Eye and Ear Infirmary who were undergoing facial aesthetic surgery with skin excision.

Methods: Five patients were injected intradermally with Autologen in one postauricular area and bovine cross-linked collagen (Zyplast) on the contralateral side. Patients were examined clinically for signs of infection, skin necrosis, or implant rejection/allergy 2, 4, and 12 weeks postinjection. Impressions and photographs of all implant sites were taken at all follow-up visits. Biopsy specimens of each implant were taken 4 and 12 weeks after injection and examined histologically for signs of integration, rejection, and resorption.

Results: All implants were well tolerated. No identifiable differences were noted in the clinical persistence of Zyplast vs Autologen. Histologically, there was more variability in the degree of fibroblast infiltration of Autologen vs Zyplast deposits.

Conclusions: Our trial suggests that autologous collagen dispersion may represent a viable alternative to bovine collagen. Clinical persistence and histologic behavior of Autologen appear to be at least as favorable as those of Zyplast, and Autologen obviates the need for allergy testing and eliminates the possibility of disease transmission.

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SURGEONS HAVE long recognized the need for a reliable material for soft-tissue replacement. Historically, surgical reconstruction was limited to the most severe cases, in which the preoperative state was so deformed that it warranted the risks of surgery and anesthesia and almost any intervention would achieve some degree of improvement. As anesthetic and surgical techniques have advanced, so have the indications for facial reconstruction. It is no longer acceptable to cover or fill a tissue void with any tissue. Surgeons now strive to replace the missing or defective tissue with analogous material, restoring structural support and/or soft-tissue volume and texture.

The science of soft-tissue fillers has advanced from the indiscriminate use of various viscous materials at the end of the 19th century to careful development of biological materials normally found in the tissue being replaced. Over the past 100 years, progress was hampered by the ill-conceived use of materials such as paraffin and adulterated silicone; the science of soft-tissue augmentation is only beginning to emerge from the shadow of these early misadventures.

The current focus of soft-tissue augmentation is loosely divided into 2 philosophies: one approach espouses the use of synthetic polymers. These are known to be relatively permanent, but their physical characteristics are not always ideal, and they remain as foreign bodies. The other approach stresses the use of biological materials either to augment directly or to elicit a reparative process that will deposit enough new tissue to fill the defect.

Autologous collagen dispersion (Autologen; Collagenesis Inc, Beverly, Mass) is derived from the patient's own skin and consists of a suspension of autologous tissue matrix predominantly composed of intact collagen fibrils. We have investigated the use of Autologen in augmenting facial soft tissue. This study examines the clinical behavior of Autologen as well as histologic findings and technical issues related to its use, with direct comparison with bovine collagen.

SUBJECTS, MATERIALS, AND METHODS

Adult patients undergoing skin resection surgery at the Facial Plastic and Reconstructive Surgery service of The New York Eye and Ear Infirmary between July 1997 and February 1998 were given the opportunity to participate in this study.

Consenting patients were tested for allergic reaction to bovine collagen in the standard fashion. Bovine collagen (0.10 mL) (Zyderm; Collagen Corp, Palo Alto, Calif) was placed intradermally with a 30-gauge needle on the volar forearm and observed for any adverse reaction for at least 30 days. Only patients who had previously been treated with bovine collagen without adverse reaction or who had negative Zyderm skin test results were allowed to participate.

At the time of surgery, hair was trimmed from the resected skin, which was then rinsed with sterile saline and frozen overnight in sterile, normal saline. The frozen specimens were then shipped by express mail to Collagenesis Inc for preparation of Autologen.

During processing of the skin, the epidermis is mechanically removed, and the dermis is minced. The resultant product is mechanically processed to generate a suspension of dermal tissue matrix, which is predominantly fibrillar, nondenatured collagen.

When the Autologen was received from the company, it was immediately refrigerated, and patients were injected within 1 week. Participating patients were anesthetized with a topical anesthetic agent (EMLA; AstraZeneca LP, Wayne, Pa) over both postauricular areas for a minimum of 20 minutes. Patients were then injected intradermally in 2 sites with Autologen (0.50 mL each) in the skin overlying the mastoid (just posterior to the postauricular crease), separated by at least 2 cm. The material was injected in small volumes serially and occupied an area no larger than a circle 10 mm in diameter; care was taken to avoid migration of the material into the skin directly in the postauricular crease. If less than 1.0 mL of Autologen was provided, the injections were divided into 2 equal aliquots. Zyplast was injected in a similar fashion on the opposite side.

The patients returned for follow-up visits at 2, 4, and 12 weeks after the initial injection. At each visit, the implant sites were inspected, and the patients were questioned regarding pain, fever, swelling, redness, and any other local or systemic symptom or sign that had developed since the injections. One implant of each type was removed (along with overlying skin) at both the 4- and 12-week visits under local anesthesia, and the wound was closed with interrupted 4-0 chromic sutures.

Standardized lateral and posterior digital photographs of the implants (with a ruler for calibration) were taken at each follow-up visit. The surface area and lateral projection of the implant were measured directly using proprietary software (Mirror 2000; Virtual Eyes Inc, Kirkland, Wash). In addition, a mold of the injection sites was fabricated with a dental epoxy (Reprosil; 3M Products, St Paul, Minn). The volumes of the implants were measured by carefully filling the depressions created by the implants in the mold with water; this was then aspirated with a 1.0-mL syringe and measured. The average of 3 measurements was recorded as the volume. Implant volume persistence was calculated as the apparent volume present (determined either by photography or molds) divided by the original volume of injection.

Pathologic specimens were fixed in formalin, sectioned, and stained with hematoxylin-eosin and with Movat stain. The sections were examined by light microscopy and were inspected for implant location and persistence, fibroblast infiltration of the implant, acute and chronic inflammation, and foreign-body inflammatory reaction. Fibroblastic activity was described as none, peri-implant, or intraimplant, based on the presence and location of the fibroblasts. Inflammatory activity was described as either none, peri-implant, or intraimplant, based on the presence and location of the inflammatory cells.

RESULTS

Ten patients were initially enrolled. Skin excised from 8 patients during surgery (2 rhytidectomies and 6 upper blepharoplasties) was shipped to the manufacturer. The skin provided was insufficient in volume to produce enough Autologen for quality testing and administration in 2 patients (both bilateral upper eyelid specimens). The dimensions of the resected specimens did not differ significantly between those patients who did not provide enough skin for Autologen and the patients providing adequate specimens. Specimen adequacy appeared to be more related to the thickness of the resected eyelid skin than to its surface area. One patient voluntarily withdrew from the study prior to injection with Autologen.

Autologen was injected in 5 patients. The volumes administered varied from 0.2 to 1.0 mL. Impressions and clinical photographs were taken for all patients at the 2- and 4-week visits (**Figure 1**); however, 2 patients were unable to complete the 12-week impressions and photography. These same 2 patients were unable to undergo the 12-week biopsy.

Data from the 2-, 4-, and available 12-week photographs (**Figure 2**) and impressions reveal similar patterns of persistence for Autologen and Zyplast. The mean percentages of material still remaining (by photography) at 2, 4, and 12 weeks for Autologen were 81.4%, 60.8%, and 42.5%, respectively. The mean percentages of material remaining (by photography) at 2, 4, and 12 weeks for Zyplast were 78.6%, 51.9%, and 31.1%, respectively. The mean percentages of material remaining (by impressions) at 2, 4, and 12 weeks for Autologen were 34.0%, 29.7%, and 15.0%, respectively. The mean percentages of material remaining (by impressions) at 2, 4, and 12 weeks for Zyplast were 42.0%, 37.7%, and 27.4%, respectively (**Table 1**). No statistically significant differences between Autologen and Zyplast were noted at any time.

Specimens were scrutinized histologically for fibroblastic and inflammatory activity (**Figure 3** and **Figure 4**). Both Autologen and Zyplast demonstrated a

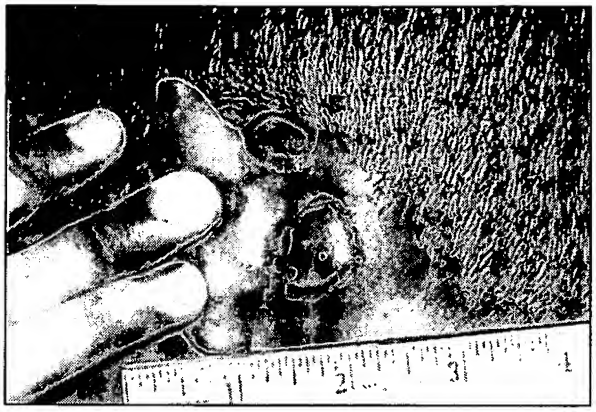

Figure 1. *Posterior digital photograph of autologous collagen dispersion (Autologen) implant. Ruler is in inches.*

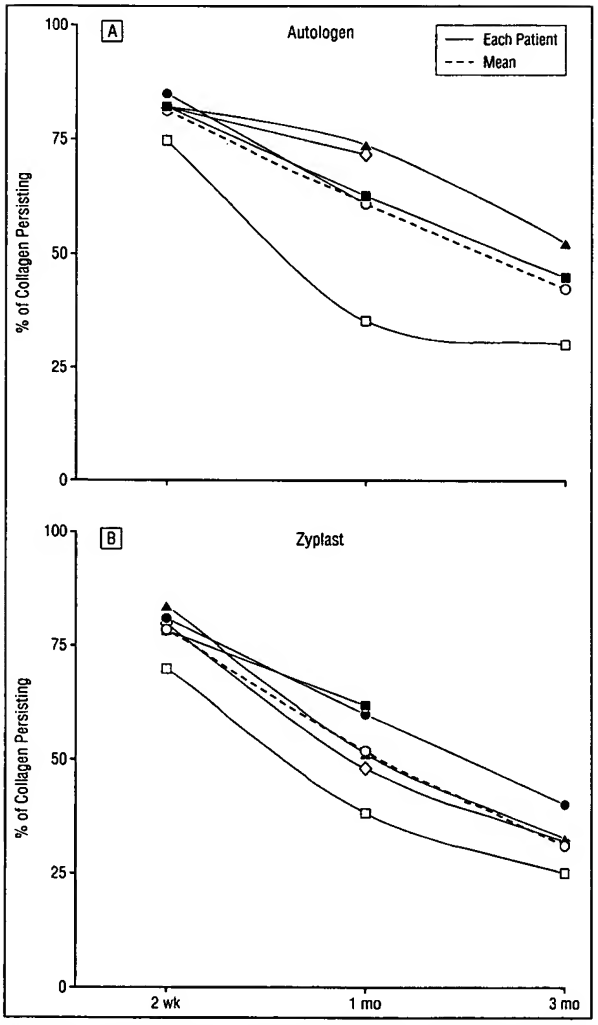

Figure 2. *Persistence of autologous collagen dispersion (Autologen) (A) and bovine cross-linked collagen (Zyplast) (B) as measured by photography over a 12-week period.*

fibroblastic reaction at the periphery of the implant. Fibroblasts invaded the core of both types of implant, although subjectively this ingrowth appeared to be greater in Autologen samples. The only foreign-body giant cell reactions were noted in 1 Zyplast implant at 4 weeks (**Table 2**).

COMMENT

Surgical treatment of tissue voids was reported as early as 600 BC and has been described over the past 2500 years for treatment of penal or wartime injuries. Early surgeons used large soft-tissue pedicle flaps to cover defects. However, in the 19th century, surgeons began to look for less invasive methods of treatment for soft-tissue deficits. Since structural support was not needed, the concept of an injectable substance to fill the soft-tissue void became a focus of scientific interest. Many workers have used a staggering number and variety of materials, often ill conceived and without any scientific grounding. These initial investigations (and frequent misadventures) have prejudiced surgeons against implantable biomaterials.

Gersuny[1] reported using paraffin injections to simulate a testicular prosthesis in 1899. Other workers quickly followed, using paraffin as a facial soft-tissue filler. Within 3 years, reports of paraffin pulmonary emboli and foreign-body granulomas had been published. Most Western surgeons abandoned paraffin injections, but paraffin continued to be used as late as the 1950s in the Far East.[2]

Silicone refers to a class of polydimethylsiloxanes that vary in their length and side chains. Silicones are odorless, tasteless, and colorless and have varying viscosities. The history of injectable silicone has been clouded by a variety of factors: silicones differing in their precise structure, manufacturing impurities, postproduction adulteration, poor experimental design, and occasionally inappropriate use. Under optimal conditions, silicone injection leads to a low-grade chronic inflammatory response. Phagocytosis, silicone migration, and recovery from distant organs have been reported with large-volume injections. Granulomas, silicone-induced pneumonitis, hepatitis, and chronic local inflammatory reactions have been reported up to several years after injection. The validity of silicone-induced collagen vascular diseases will continue to be debated for decades to come.

The most common soft-tissue alloplast currently used is polytetrafluoroethylene (Gore-Tex; W. L. Gore & Associates, Flagstaff, Ariz). Gore-Tex has been used successfully for the past 7 to 8 years for facial soft-tissue deficit repair and for the treatment of lip augmentation with limited complications. However, the possibility of infection and extrusion affects the use of any synthetic implant, and no implant currently in use accurately approximates the pliancy, elasticity, and density of skin. More physiological solutions to the problem of soft-tissue defects are currently in use. However, all of these are limited by the one feature for which nonbiologic materials are indisputably superior: implant permanence.

Biologic fillers attempt to replace or augment dermis or subdermal tissue. Autologous fat has been advocated as an easily obtained material for augmentation. However, persistence of injected fat is widely variable,[3] from 20% to 80%, and may be better if injected into muscle.[4] Lipocytic dermal augmentation, described by Coleman et al,[5] theoretically uses the postwounding reparative response to gen-

Table 1. Persistence of Initial Injection of Autologous Collagen Dispersion (Autologen) and Bovine Cross-Linked Collagen (Zyplast) Over 12 Weeks as Measured by Photography and Molds

Time After Injection, wk	Persistence by Photography, %		Persistence by Molds, %	
	Autologen	Zyplast	Autologen	Zyplast
2	81.4	78.6	34.0	42.0
4	60.8	51.9	29.7	37.7
12	42.5	31.1	15.0	27.4

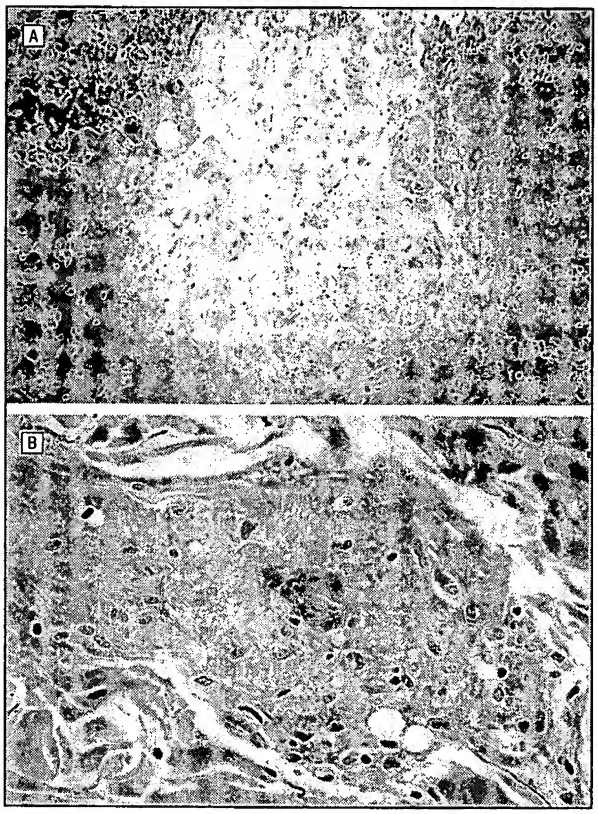

Figure 3. *Microscopic views of autologous collagen dispersion (Autologen) implant 12 weeks after injection (A: hematoxylin-eosin, original magnification ×10; B: Movat stain, original magnification ×40).*

erate soft-tissue augmentation. Fibrel (Mentor Corp, Goleta, Calif) adds the patient's plasma (as a source of fibrinogen) to porcine gelatin (as a carrier to localize the inflammatory reaction) and ϵ-aminocaproic acid (as an inhibitor of fibrinolysis). The ensuing coagulation cascade is thought to lead to fibrin and later collagen deposition. Isologen (Isologen Technologies, Metuchen, NJ) also relies on a competent inflammatory and reparative response to wounding to generate sufficient collagen to achieve adequate augmentation. Isologen is a logical continuation of the subcision technique described by Orentreich,[6] whereby lysis of subdermal scar tissue creates an environment in which the body's natural reparative process generates sufficient scar tissue to fill a tissue void. Isologen directly adds cultured fibroblasts to conduct this process. However, long-term studies on Isologen persistence have not yet been published.

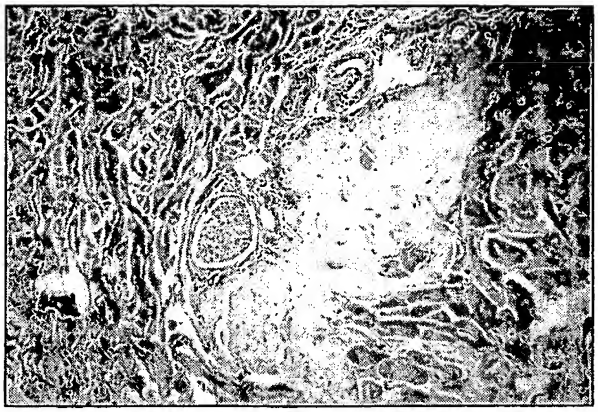

Figure 4. *Microscopic view of Zyplast implant 12 weeks after injection (hematoxylin-eosin, original magnification ×10).*

Table 2. Histologic Findings in Autologous Collagen Dispersion (Autologen) and Bovine Cross-Linked Collagen (Zyplast) Specimens 4 and 12 Weeks After Injection*

Patient	Fibroblast Location		Inflammation	
	4 wk	12 wk	4 wk	12 wk
		Autologen		
1	Periphery	...	None	...
2	Periphery	Periphery	None	Periphery
3	Periphery	...	None	...
4	Core	Periphery	Periphery	Periphery
5	Core	Periphery	Periphery	Periphery
		Zyplast		
1	Periphery	...	None	...
2	Core	Periphery	Periphery	Periphery
3	Core	...	Foreign-body giant cells	...
4	Periphery	Periphery	Periphery	None
5	Periphery	Core	None	Periphery

**Ellipses indicate data not available.*

lished. All of these techniques are founded on the supposition that the body will better tolerate autologous tissue with less antigenic stimulation and thus be subject to less resorption. However, issues related to the delivery system and placement may be more significant. Definitive long-term studies are needed.

Bovine collagen is the simplest and most commonly used biologic filler material; because of this, it remains the criterion standard for injectable biologic fillers. It is readily available in large quantities, easily placed, and generally well tolerated. However, a small percentage (approximately 3%) of patients will display allergic sensitivity on the collagen skin test; a much smaller proportion of patients (approximately 1%) can develop allergic sensitivity during treatment. An association of bovine collagen administration with autoimmune disease has never been shown conclusively. However, the most significant drawback to bovine collagen is the relatively rapid (3-4 months) and inexorable resorption by the body and loss of clinical effect. Because of its ease in use and administration, bovine collagen currently enjoys its status as the most commonly used injectable filler.

We have investigated the use of injectable Autologen for soft-tissue augmentation. Autologen and its homologous counterpart (Dermalogen) are derived from human dermis. Autologen is a dispersion of dermal matrix shown to contain intact collagen of types I, III, and VI; elastic fibers; fibronectin; and glycosaminoglycans. This material is easily injected into the dermis using a 30-gauge needle in a fashion similar to bovine collagen. Unlike bovine collagen, which is treated to remove the telopeptide units (reducing immunogenicity and leading to loss of collagen fiber alignment), Autologen and Dermalogen contain only human, nonallergenic proteins; the tight alignment of the collagen fibers and the absence of any xenoproteins were believed to reduce the resultant inflammatory response to the implant and reduce subsequent resorption. The obvious advantage of Autologen is its autologous nature. There is no potential for allergic reaction or donor-to-recipient disease transmission, and the concept of Autologen is very attractive to patients undergoing skin-excision procedures. Those patients not undergoing facelift, abdominoplasty, breast reduction, or other skin-excision procedures must submit themselves to a skin-harvesting procedure. In response to this issue, the manufacturer has introduced Dermalogen, which is derived from skin obtained from tissue banks. While eliminating the need for skin excision, this material is homologous and has features that are theoretically somewhere in the spectrum between homologous (bovine collagen) and autologous (Autologen) material.

Autologen has been used with good clinical results for treatment of vocal fold paralysis[7] and facial rhytids,[8,9] but long-term, objective studies have not been performed. In our study, we did not observe a statistically significant difference between Autologen and Zyplast in implant persistence over a 12-week period. However, when examining the implant survival curves, there appears to be a slight trend toward longer persistence of Autologen than Zyplast. Our small sample size and relatively short follow-up may have masked a clinically significant difference between these 2 materials. Larger studies with longer follow-up periods are in development.

In our study, digital photography appeared to be a better method than surface molds to measure the size of the implant. The molds required the measurement of a liquid (water) filling the depression created by the implant. The meniscus formed by this water made precise measurements impossible. Of course, both methods measure only a portion of the implant, which is a 3-dimensional structure with displacement of deep as well as superficial tissues. Laser scanners or ultrasonic devices may be better tools for measuring all surfaces of dermal implants, but high-resolution digital photography allowed us to monitor the clinically relevant (external) effect of the implant.

Histologically, there was no significant difference in the degree of fibroblast infiltration of inflammatory response. Because of the small sample size, no statistical conclusions can be drawn in regard to the clinical differences between Autologen and Zyplast. However, it is unclear whether intact collagen fibers in an altered milieu can avoid eliciting a resorptive process. The degree of protection offered by and the behavior elicited by the association of collagen with various ground substances certainly may affect the body's response to injection of Autologen. Additionally, the qualities of the skin at the injection site also affect the subsequent behavior of the implant. Clinicians have long recognized that injectable fillers placed in sites subject to significant muscular activity (eg, perioral complex and melolabial folds) undergo a greater degree of resorption than those at other sites. Finally, the tensile and elastic characteristics of skin may also affect injectable implant behavior; replacement of volume in an area of chronoaged skin and skin with less elasticity may be more successful than augmentation of younger, more elastic, skin.

CONCLUSIONS

Autologous dermal matrix dispersion (Autologen) can be readily produced from the skin that is usually discarded during skin-excision procedures. Autologen is very well tolerated and clinically persists at least as well as Zyplast. There is no need for pretreatment allergy testing, and there is no risk of disease transmission or induction. Further work on Autologen and its homologous counterpart Dermalogen will elucidate the role that these materials will play in replacing facial soft tissue.

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US005618551A

United States Patent [19]

Tardy et al.

[11] Patent Number: 5,618,551

[45] Date of Patent: Apr. 8, 1997

[54] **BIOCOMPATIBLE BIORESORBABLE AND NON-TOXIC ADHESIVE COMPOSITION FOR SURGICAL USE**

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[73] Assignee: **Imedex**, Chaponost, France

[21] Appl. No.: **376,185**

[22] Filed: **Jan. 20, 1995**

[30] **Foreign Application Priority Data**

Jan. 24, 1994 [FR] France 94 00715

[51] **Int. Cl.**[6] **A61L 25/00**

[52] **U.S. Cl.** **424/426**; 424/443; 424/444; 424/445; 424/484

[58] **Field of Search** 424/426, 443, 424/444, 445, 484

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Primary Examiner—Thurman K. Page
Assistant Examiner—Sharon Howard
Attorney, Agent, or Firm—Larson & Taylor

[57] **ABSTRACT**

The invention relates to a biocompatible, bioresorbable and non-toxic adhesive composition for surgical use, for the bonding, in particular, of biological tissues to one another or in an implanted biomaterial, characterized in that it comprises a reactive acidic solution of non-crosslinked and potentially crosslinkable collagen or gelatin modified by oxidative cleavage, at a concentration which is preferably between approximately 5 and 30 by weight. It also relates to reactive acidic solutions and powders based on non-crosslinked collagen or gelatin modified by oxidative cleavage which are used as intermediate products in the preparation of the above-mentioned composition, and to the process for their preparation. It also relates to adhesive kits which comprise, on the one hand, the above-mentioned reactive acidic solution and, on the other hand, a neutralizing solution and which are intended for extemporaneous mixing. Finally it relates to a method of application of the adhesive, composition according to the invention. The invention is particularly useful in the areas of adhesion, haemostasis, leaktightness with respect to liquids or gases, cicatrization, filling, avoiding adhesion in surgery, embolization, as a local system for release of medicamentary active principles, etc.

26 Claims, 1 Drawing Sheet

US007247314B2

(12) **United States Patent**
Hnojewyj et al.

(10) **Patent No.: US 7,247,314 B2**
(45) **Date of Patent: Jul. 24, 2007**

(54) **BIOCOMPATIBLE MATERIAL COMPOSITION ADAPTABLE TO DIVERSE THERAPEUTIC INDICATIONS**

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(73) Assignee: **Neomend, Inc**, Irvine, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **11/002,837**

(22) Filed: **Dec. 2, 2004**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**

A61K 47/42	(2006.01)
C08L 89/00	(2006.01)
C12N 11/04	(2006.01)

(52) **U.S. Cl.** **424/426**; 424/486; 435/182; 525/54.1; 530/815

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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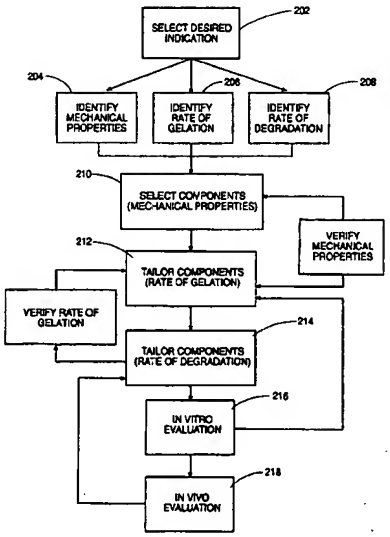
(74) *Attorney, Agent, or Firm*—Ryan, Kromholz, and Manion S.C.

(57) **ABSTRACT**

A biocompatible material genus serves as the foundation for multiple material composition species, each adapted to a specific therapeutic indication.

18 Claims, 5 Drawing Sheets



US005410016A

United States Patent [19]

Hubbell et al.

[11] Patent Number: 5,410,016

[45] Date of Patent: Apr. 25, 1995

[54] **PHOTOPOLYMERIZABLE BIODEGRADABLE HYDROGELS AS TISSUE CONTACTING MATERIALS AND CONTROLLED-RELEASE CARRIERS**

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[21] Appl. No.: **22,687**

[22] Filed: **Mar. 1, 1993**

Related U.S. Application Data

[60] Continuation-in-part of Ser. No. 843,485, Feb. 28, 1992, abandoned, Ser. No. 598,880, Oct. 15, 1990, and Ser. No. 740,703, Aug. 5, 1991, which is a division of Ser. No. 598,880, Aug. 5, 1991.

[51] **Int. Cl.**[6] **C08G 63/08**; C08G 67/00; A61K 9/58

[52] **U.S. Cl.** **528/354**; 128/898; 424/426; 424/489; 525/54.1; 525/54.2; 525/408; 525/413; 525/415; 514/772.1; 514/772.3; 514/773; 514/777; 528/361

[58] **Field of Search** 424/426, 489; 514/772.1, 772.3, 773, 777; 525/54.1, 54.2, 408, 413, 415; 528/354, 361; 128/898

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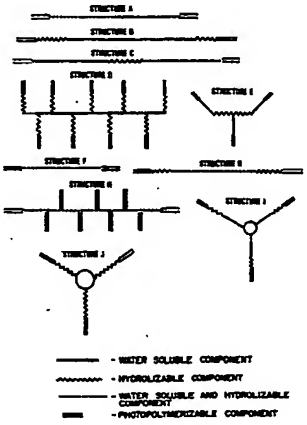
[57] **ABSTRACT**

Hydrogels of polymerized and crosslinked macromers comprising hydrophilic oligomers having biodegradable monomeric or oligomeric extensions, which biodegradable extensions are terminated on free ends with end cap monomers or oligomers capable of polymerization and cross linking are described. The hydrophilic core itself may be degradable, thus combining the core and extension functions. Macromers are polymerized using free radical initiators under the influence of long wavelength ultraviolet light, visible light excitation or thermal energy. Biodegradation occurs at the linkages within the extension oligomers and results in fragments which are non-toxic and easily removed from the body. Preferred applications for the hydrogels include prevention of adhesion formation after surgical procedures, controlled release of drugs and other bioactive species, temporary protection or separation of tissue surfaces, adhering of sealing tissues together, and preventing the attachment of cells to tissue surfaces.

23 Claims, 9 Drawing Sheets



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(54) Title: PHOTOPOLYMERIZABLE BIODEGRADABLE HYDROGELS AS TISSUE CONTACTING MATERIALS AND CONTROLLED-RELEASE CARRIERS

(57) Abstract

Hydrogels of polymerized and crosslinked macromers comprising hydrophilic oligomers having biodegradable monomeric or oligomeric extensions, which biodegradable extensions are terminated on free ends with end cap monomers or oligomers capable of polymerization and cross linking are described. The hydrophilic core itself may be degradable, thus combining the core and extension functions. Macromers are polymerized using free radical initiators under the influence of long wavelength ultraviolet light, visible light excitation or thermal energy. Biodegradation occurs at the linkages within the extension oligomers and results in fragments which are non-toxic and easily removed from the body. Preferred applications for the hydrogels include prevention of adhesion formation after surgical procedures, controlled release of drugs and other bioactive species, temporary protection or separation of tissue surfaces, adhering of sealing tissues together, and preventing the attachment of cells to tissue surfaces.

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PHOTOPOLYMERIZABLE BIODEGRADABLE HYDROGELS AS TISSUE CONTACTING MATERIALS AND CONTROLLED-RELEASE CARRIERS

Field of the Invention

The present invention relates to photopolymerizable biodegradable hydrogels for use as tissue adhesives and in controlled drug delivery.

Background of the Invention

This is a continuation-in-part of U.S. Patent Application No. 07/843,485, filed February 28, 1992, entitled "Photopolymerizable Biodegradable Hydrogels as Tissue Contacting Materials and Controlled Release Carriers" by Jeffrey A. Hubbell, Chandrashekhar P. Pathak, and Amarpreet S. Sawhney.

Hydrogels as controlled-release carriers

Biodegradable hydrogels can be carriers for biologically active materials such as hormones, enzymes, antibiotics, antineoplastic agents, and cell suspensions. Temporary preservation of functional properties of a carried species, as well as controlled release of the species into local tissues or systemic circulation, are possible. Proper choice of hydrogel macromers can produce membranes with a range of permeability, pore sizes and degradation rates suitable for a variety of applications in surgery, medical diagnosis and treatment.

Adhesives and sealers

Fibrin gels have been used extensively in Europe as sealants and adhesives in surgery (Thompson *et al.*, 1988, "Fibrin Glue: A review of its preparation, efficacy, and adverse effects as a topical hemostat," *Drug Intell. and Clin. Pharm.*, 22:946; Gibble *et al.*, 1990, (1990), "Fibrin glue: the perfect operative sealant?" *Transfusion*, 30(8):741). However, they have not been used extensively in the United States

due to concerns relating to disease transmission from blood products. Synthetic polymers have been explored as adhesives (Lipatova, 1986, "Medical polymer adhesives," *Advances in Polymer Science* 79:65-93), but these materials have been associated with local inflammation, cytotoxicity, and poor biocompatibility.

Prevention of postoperative adhesions.

Formation of post-surgical adhesions involving organs of the peritoneal cavity and the peritoneal wall is a frequent and undesirable result of abdominal surgery. Surgical trauma to the tissue caused by handling and drying results in release of a serosanguinous (proteinaceous) exudate which tends to collect in the pelvic cavity (Holtz, G., 1984). If the exudate is not absorbed or lysed within this period it becomes ingrown with fibroblasts, and subsequent collagen deposition leads to adhesion formation.

Numerous approaches to elimination of adhesion formation have been attempted, with limited success in most cases. Approaches have included lavage of the peritoneal cavity, administration of pharmacological agents, and the application of barriers to mechanically separate tissues. For example, Boyers *et al.*, (1988) "Reduction of postoperative pelvic adhesions in the rabbit with Gore-Tex surgical membrane," *Fertil. Steril.*, 49:1066, examined Gore-Tex surgical membranes in the prevention of adhesions. For a review of adhesion prevention, see Holtz (1984) "Prevention and management of peritoneal adhesions," *Fertil. Steril.*, 41:497-507. However, none of these approaches has been cost effective and effective in *in vivo* studies.

Solutions of Poloxamer 407 have been used for the treatment of adhesions, with some success. Poloxamer is a copolymer of ethylene oxide and propylene oxide and is soluble in water; the solutions are liquids at

room temperature. Steinleitner *et al.* (1991) "Poloxamer 407 as an Intraperitoneal Barrier Material for the Prevention of Postsurgical Adhesion Formation and Reformation in Rodent Models for Reproductive Surgery," *Obstetrics and Gynecology*, 77(1):48 and Leach *et al.* (1990) "Reduction of postoperative adhesions in the rat uterine horn model with poloxamer 407, *Am. J. Obstet. Gynecol.*, 162(5):1317, examined Poloxamer solutions in peritoneal adhesion models and observed statistically significant reductions in adhesions; however, they were unable to eliminate adhesions, perhaps because of limited adhesion and retention on the injury site.

Oxidized regenerated cellulose has been used extensively to prevent adhesions and is an approved clinical product, trade-named Interceed TC7. This barrier material has been shown to be somewhat effective in rabbits (Linsky *et al.*, 1987 "Adhesion reduction in a rabbit uterine horn model using TC-7," *J. Reprod. Med.*, *32*:17; Diamond *et al.*, 1987 "Pathogenesis of adhesions formation/reformation: applications to reproductive surgery," *Microsurgery*, 8:103) and in humans (Interceed (TC7) *Adhesion Barrier Study Group*, 1989). It was shown to be more effective if pretreated with heparin, but was still unable to completely eliminate adhesions (Diamond *et al.*, 1991 "Synergistic effects of INTERCEED(TC7) and heparin in reducing adhesion formation in the rabbit uterine horn model," *Fertility and Sterility*, 55(2):389).

In summary, several lavage/drug/material approaches have been explored, but none of these approaches has been able to eliminate adhesions. An ideal material barrier would not evoke an adhesion response itself, stay in place without suturing (Holtz *et al.*, 1982 "Adhesion induction by suture of varying tissue reactivity and caliber," *Int. J. Fert.*, 27:134), degrade over a few weeks' time, effectively

reduce adhesions to very low extent, and be capable of delivering a drug to the local site of application for several days' time. None of the approaches developed and described to date meet these requirements.

Synthetic biodegradable polymers

The field of biodegradable polymers has developed rapidly since the synthesis and biodegradability of polylactic acid was first reported by Kulkarni *et al.*, 1966 "Polylactic acid for surgical implants," *Arch. Surg.*, 93:839. Several other polymers are known to biodegrade, including polyanhydrides and polyorthoesters, which take advantage of labile backbone linkages, as reported by Domb *et al.*, 1989 *Macromolecules*, 22:3200; Heller *et al.*, 1990 *Biodegradable Polymers as Drug Delivery Systems*, Chasin, M. and Langer, R., Eds., Dekker, New York, 121-161. Since it is desirable to have polymers that degrade into naturally occurring materials, polyaminoacids have been synthesized, as reported by Miyake *et al.*, 1974, for *in vivo* use. This was the basis for using polyesters (Holland *et al.*, 1986 *Controlled Release*, 4:155-180) of α-hydroxy acids (*viz.*, lactic acid, glycolic acid), which remain the most widely used biodegradable materials for applications ranging from closure devices (sutures and staples) to drug delivery systems (U.S. Patent No. 4,741,337 to Smith *et al.*; Spilizewski *et al.*, 1985 "The effect of hydrocortisone loaded poly(dl-lactide) films on the inflammatory response," *J. Control. Rel.* 2:197-203).

The time required for a polymer to degrade can be tailored by selecting appropriate monomers. Differences in crystallinity also alter degradation rates. Due to the relatively hydrophobic nature of these polymers, actual mass loss only begins when the oligomeric fragments are small enough to be water

soluble. Hence, initial polymer molecular weight influences the degradation rate.

Degradable polymers containing water-soluble polymer elements have been described. Sawhney et al., (1990) "Rapidly degraded terpolymers of dl-lactide, glycolide, and ϵ-caprolactone with increased hydrophilicity by copolymerization with polyethers," *J. Biomed. Mater. Res.* 24:1397-1411, copolymerized lactide, glycolide and ϵ-caprolactone with PEG to increase its hydrophilicity and degradation rate. U.S. Patent No. 4,716,203 to Casey et al. (1987) synthesized a PGA-PEG-PGA block copolymer, with PEG content ranging from 5-25% by mass. U.S. Patent No. 4,716,203 to Casey et al. (1987) also reports synthesis of PGA-PEG diblock copolymers, again with PEG ranging from 5-25%. U.S. Patent No. 4,526,938 to Churchill et al. (1985) described noncrosslinked materials with MW in excess of 5,000, based on similar compositions with PEG; although these materials are not water soluble. Cohn et al. (1988) *J. Biomed. Mater. Res.* 22:993-1009 described PLA-PEG copolymers that swell in water up to 60%; these polymers also are not soluble in water, and are not crosslinked. The features that are common to these materials is that they use both water-soluble polymers and degradable polymers, and that they are insoluble in water, collectively swelling up to about 60%.

Degradable materials of biological origin are well known, for example, crosslinked gelatin. Hyaluronic acid has been crosslinked and used as a degradable swelling polymer for biomedical applications (U.S. Patent No. 4,987,744 to della Valle et al., U.S. Patent 4,957,744 to Della Valle *et al.* (1991) "Surface modification of polymeric biomaterials for reduced thrombogenicity," *Polym. Mater. Sci. Eng.*, 62:731-735]).

Use of biodegradable materials for controlled drug release.

Most hydrophilic drugs are mechanically dispersed as suspensions within solutions of biodegradable polymers in organic solvents. Protein and enzyme molecular conformations are frequently different under these circumstances than they would be in aqueous media. An enzyme dispersed in such a hydrophobic matrix is usually present in an inactive conformation until it is released into the surrounding aqueous environment subsequent to polymer degradation. Additionally, some proteins may be irreversibly denatured by contact with organic solvents used in dispersing the protein within the polymer.

Polymer synthesis, degradation and local synthesis

Rapidly-degrading polymers currently suggested for short-term macromolecular drug release may raise local concentrations of potentially hazardous acidic degradation byproducts. Further, all biodegradable synthetic polymers reported thus far can only be processed in organic solvents and all biodegradable polymers are synthesized under conditions which are not amenable to polymerization *in vivo*. Thus, it has not been possible to make implantable materials as precisely conformed barriers, shaped articles, or membranes capable of delivering bioactive materials to the local tissue.

It is therefore an object of the present invention to provide hydrogels which are biocompatible, biodegradable, and can be rapidly formed by polymerization *in vivo*.

It is a further object of the present invention to provide a macromer solution which can be administered during surgery or outpatient procedures and polymerized as a tissue adhesive, tissue encapsulating medium, tissue support, or drug delivery medium.

It is a still further object of the present invention to provide a macromer solution which can be polymerized *in vivo* in a very short time frame and in very thin, or ultrathin, layers.

Summary of the Invention

Disclosed herein are biocompatible, biodegradable, polymerizable and at least substantially water soluble macromers, having a variety of uses *in vivo*. The macromers include at least one water soluble region, at least one region which is biodegradable, usually by hydrolysis, and at least two free radical-polymerizable regions. The regions can, in some embodiments, be both water soluble and biodegradable. The macromers are polymerized by exposure of the polymerizable regions to free radicals generated, for example, by photosensitive chemicals and dyes.

An important aspect of the macromers are that the polymerizable regions are separated by at least one degradable region to facilitate uniform degradation *in vivo*.. There are several variations of these polymers. For example, the polymerizable regions can be attached directly to degradable extensions or indirectly via water soluble nondegradable sections so long as the polymerizable regions are separated by a degradable section. For example, if the macromer contains a simple water soluble region coupled to a degradable region, one polymerizable region may be attached to the water soluble region and the other attached to the degradable extension or region. In another embodiment, the water soluble region forms the central core of the macromer and has at least two degradable regions attached to the core. At least two polymerizable regions are attached to the degradable regions so that, upon degradation, the polymerizable regions, particularly in the polymerized gel form, are

separated. Conversely, if the central core of the macromer is formed by a degradable region, at least two water soluble regions can be attached to the core and polymerizable regions attached to each water soluble region. The net result will be the same after gel formation and exposure to *in vivo* degradation conditions. In still another embodiment, the macromer has a water soluble backbone region and a degradable region affixed to the macromer backbone. At least two polymerizable regions are attached to the degradable regions, so that they are separated upon degradation, resulting in gel product dissolution. In a further embodiment, the macromer backbone is formed of a nondegradable backbone having water soluble regions as branches or grafts attached to the degradable backbone. Two or more polymerizable regions are attached to the water soluble branches or grafts. In another variation, the backbone may be star shaped, which may include a water soluble region, a biodegradable region or a water soluble region which is also biodegradable. In this general embodiment, the star region contains either water soluble or biodegradable branches or grafts with polymerizable regions attached thereto. Again, the polymerizable regions must be separated at some point by a degradable region.

Examples of these macromers are PEG-oligoglycolyl-acrylates. The choice of appropriate end caps permits rapid polymerization and gelation; acrylates were selected because they can be polymerized using several initiating systems, e.g., an eosin dye, by brief exposure to ultraviolet or visible light. The poly(ethyleneglycol) or PEG central structural unit (core) was selected on the basis of its high hydrophilicity and water solubility, accompanied by excellent biocompatibility. A short oligo or poly(α-hydroxy acid), such as polyglycolic

acid, was selected as a preferred chain extension because it rapidly degrades by hydrolysis of the ester linkage into glycolic acid, a harmless metabolite. Although highly crystalline polyglycolic acid is insoluble in water and most common organic solvents, the entire macromer is water-soluble and can be rapidly gelled into a biodegradable network while in contact with aqueous tissue fluids. Such networks can be used to entrap and homogeneously disperse water-soluble drugs and enzymes and to deliver them at a controlled rate. Further, they may be used to entrap particulate suspensions of water-insoluble drugs. Other preferred chain extensions are polylactic acid, polycaprolactone, polyorthoesters, and polyanhydrides. Polypeptides may also be used. Such "polymeric" blocks should be understood to include timeric, trimeric, and oligomeric blocks.

These materials are particularly useful for controlled drug delivery, especially of hydrophilic materials, since the water soluble regions of the polymer enable access of water to the materials entrapped within the polymer. Moreover, it is possible to polymerize the macromer containing the material to be entrapped without exposing the material to organic solvents. Release may occur by diffusion of the material from the polymer prior to degradation and/or by diffusion of the material from the polymer as it degrades, depending upon the characteristic pore sizes within the polymer, which is controlled by the molecular weight between crosslinks and the crosslink density. Deactivation of the entrapped material is reduced due to the immobilizing and protective effect of the gel and catastrophic burst effects associated with other controlled-release systems are avoided. When the entrapped material is an enzyme, the enzyme can be exposed to substrate while the enzyme is entrapped, provided the gel proportions are chosen to

allow the substrate to permeate the gel. Degradation of the polymer facilitates eventual controlled release of free macromolecules *in vivo* by gradual hydrolysis of the terminal ester linkages.

An advantage of these macromers are that they can be polymerized rapidly in an aqueous surrounding. Precisely conforming, semi-permeable, biodegradable films or membranes can thus be formed on tissue *in situ* to serve as biodegradable barriers, as carriers for living cells or other biologically active materials, and as surgical adhesives. In a particularly preferred embodiment, the macromers are applied to tissue having bound thereto an initiator, and polymerized to form ultrathin coatings. This is especially useful in forming coatings on the inside of tissue lumens such as blood vessels where there is a concern regarding restenosis, and in forming tissue barriers during surgery which thereby prevent adhesions from forming.

Examples demonstrate the use of these macromers and polymers for the prevention of postoperative surgical adhesions in rat cecum and rabbit uterine horn models. The polymer shows excellent biocompatibility, as seen by a minimal fibrous overgrowth on implanted samples. Hydrogels for the models were gelled in situ from water-soluble precursors by brief exposure to long wavelength ultraviolet (LWUV) light, resulting in formation of an interpenetrating network of the hydrogel with the protein and glycosaminoglycan components of the tissue. The degradable hydrogel was very effective, both by itself and in combination with tPA, in preventing adhesions.

Brief Description of the Drawings

Figure 1 shows schematically illustrated macromers of the present invention where _____ is a

water soluble core such as PEG; ~~~~~ is a hydrolyzably degradable extension such as a polyglycolide; ====== is a polymerizable end cap or side chain such as an acrylate; and ------ is a water-soluble and hydrolyzable portion such as a hyaluronate.

Figure 1A shows the degree of photopolymerization (dp) calculated and found by NMR.

Figure 2A shows Human foreskin fibroblasts cultured for six hours on glass coverslips coated with PEG 18.5K-glycolide diacrylate (18.5KG).

Figure 2B shows Human foreskin fibroblasts cultured for six hours on glass coverslips not coated with PEG.

Figure 3A shows the release of BSA from a PEG 1K (1000 molecular weight PEG) glycolide diacrylate with glycolide extensions (1 KG) hydrogel into PBS.

Figure 3B shows release of lysozyme from PEG 18.5K-DL-lactide tretraacrylate (18.5KL) into PBS.

Figure 4A shows release of active recombinant tPA from a PEG 1K lactide diacrylate (1KL) hydrogel.

Figure 4B shows release of active recombinant t-PA from PEG 4K glycolide diacrylate (4KG) hydrogel.

Figure 4C shows release of active recombinant tPA from a PEG 18.5K-glycolide diacrylate (18.5KG) hydrogel into PBS.

Figure 5A is a superior view of rabbit uterine horn used as a control. Distorted horn anatomy with 66% adhesions is evident. The horns are folded upon themselves.

Figure 5B is a superior view of rabbit uterine horn treated with a photopolymerized biodegradable hydrogel, PEG 18.5KL. Horn anatomy is normal, with no adhesion bands visible.

Figure 6A is an environmental scanning electron micrograph (ESEM) of an untreated blood vessel following trauma.

Figure 6B is an ESEM of a polymer coated blood vessel following trauma.

Description of the Preferred Embodiments

Disclosed herein are water soluble, biodegradable polymers formed from macromers containing both water soluble regions as well as biodegradable regions and at least two regions which are polymerizable by free radical initiation, preferably by photopolymerization using visible or long wavelength ultraviolet radiation.

The macromers.

In general terms, the macromers are polymers that are soluble in aqueous solutions, or nearly aqueous solutions, such as water with added dimethylsulfoxide. They have three components including a biodegradable region, preferably hydrolyzable under *in vivo* conditions, a water soluble region, and at least two polymerizable regions. Examples of these structures are shown in Figure 1.

Structure A in Figure 1 shows a macromer having a water soluble region (_____), a water soluble and degradable component (------) appended to one another. Each has a polymerizable end cap (======). Structure B shows a major water soluble component or core region (_____) extended at either end by a degradable or hydrolyzable component (~~~~~~) and terminated by, at either end, a polymerizable component (======). Structure C shows a central degradable or hydrolyzable component (~~~~~~) bound to a water soluble component (_____) capped at either end by a polymerizable component (======). Structure D shows a central water soluble component (_____) with numerous branches of hydrolyzable components (~~~~~~), each hydrolyzable component being capped with a polymerizable component (======). Structure E shows a central biodegradable, hydrolyzable component (~~~~~~) with three water

soluble branches (_____), each water soluble branch being capped by a polymerizable component (======). Structure F shows a long central water soluble and hydrolyzable component (------), each end being capped by a polymerizable component (======). Structure G shows a central water soluble and hydrolyzable component (------) capped at both ends by a hydrolyzable component (~~~~~~), each hydrolyzable component being capped by a polymerizable component (======). Structure H shows a central water soluble and degradable or hydrolyzable component (------) with end caps or branches of a polymerizable component (======). Structure I shows a central water soluble component (_____) in circular form with water soluble branches extended by a hydrolyzable component (~~~~~~) capped by a polymerizable component (======). Lastly, Structure J in Figure 1 shows a circular water soluble core component (_____) with degradable branches (~~~~~~), each being capped by a polymerizable component (~~~~~~).

The various structures shown in Figure 1 are exemplary only. Those skilled in the art will understand many other possible combinations which could be utilized for the purposes of the present invention.

Used herein is the term "at least substantially water soluble." This is indicative that the solubility should be at least about 1 g/100 ml of aqueous solution or in aqueous solution containing small amounts of organic solvent, such as dimethylsulfoxide. By the term "polymerizable" is meant that the regions have the capacity to form additional covalent bonds resulting in macromer interlinking, for example, carbon-carbon double bonds of acrylate-type molecules. Such polymerization is characteristically initiated by free-radical formation, for example, resulting from photon

absorption of certain dyes and chemical compounds to ultimately produce free-radicals.

In a preferred embodiment, a hydrogel begins with a biodegradable, polymerizable, macromer including a core, an extension on each end of the core, and an end cap on each extension. The core is a hydrophilic polymer or oligomer; each extension is a biodegradable polymer or oligomer; and each end cap is an oligomer, dimer or monomer capable of cross-linking the macromers. In a particularly preferred embodiment, the core includes hydrophilic poly(ethylene glycol) oligomers of molecular weight between about 400 and 30,000 Da; each extension includes biodegradable poly (α-hydroxy acid) oligomers of molecular weight between about 200 and 1200 Da; and each end cap includes an acrylate-type monomer or oligomer (i.e., containing carbon-carbon double bonds) of molecular weight between about 50 and 200 Da which are capable of cross-linking and polymerization between copolymers. More specifically, a preferred embodiment incorporates a core consisting of poly(ethylene glycol) oligomers of molecular weight between about 8,000 and 10,000 Da; extensions consisting of poly(lactic acid) oligomers of molecular weight about 250 Da; and end caps consisting acrylate moieties of about 100 Da molecular weight.

Those skilled in the art will recognize that oligomers of the core, extensions and end caps may have uniform compositions or may be combinations of relatively short chains or individual species which confer specifically desired properties on the final hydrogel while retaining the specified overall characteristics of each section of the macromer. The lengths of oligomers referred to herein may vary from two mers to many, the term being used to distinguish subsections or components of the macromer from the complete entity.

Water soluble regions.

In preferred embodiments, the core water soluble region can consist of poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propyleneoxide) block copolymers, polysaccharides or carbohydrates such as hyaluronic acid, dextran, heparan sulfate, chondroitin sulfate, heparin, or alginate, proteins such as gelatin, collagen, albumin, ovalbumin, or polyamino acids.

Biodegradable regions.

The biodegradable region is preferably hydrolyzable under *in vivo* conditions. For example, hydrolyzable group may be polymers and oligomers of glycolide, lactide, ϵ-caprolactone, other hydroxy acids, and other biologically degradable polymers that yield materials that are non-toxic or present as normal metabolites in the body. Preferred poly(α-hydroxy acid)s are poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid). Other useful materials include poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines) and poly(phosphoesters). Polylactones such as poly(ϵ-caprolactone), poly(ϵ-caprolactone), poly(δ-valerolactone) and poly(gamma-butyrolactone), for example, are also useful. The biodegradable regions may have a degree of polymerization ranging from one up to values that would yield a product that was not substantially water soluble. Thus, monomeric, dimeric, trimeric, oligomeric, and polymeric regions may be used.

Biodegradable regions can be constructed from polymers or monomers using linkages susceptible to biodegradation, such as ester, peptide, anhydride, orthoester, phosphazine and phosphoester bonds.

Polymerizable regions.

The polymerizable regions are preferably polymerizable by photoinitiation by free radical generation, most preferably in the visible or long wavelength ultraviolet radiation. The preferred polymerizable regions are acrylates, diacrylates, oligoacrylates, methacrylates, dimethacrylates, oligomethoacrylates, or other biologically acceptable photopolymerizable groups.

Other initiation chemistries may be used besides photoinitiation. These include, for example, water and amine initiation schemes with isocyanate or isothiocyanate containing macromers used as the polymerizable regions.

Photoinitiators and/or Catalysts.

Useful photoinitiators are those which can be used to initiate by free radical generation polymerization of the macromers without cytotoxicity and within a short time frame, minutes at most and most preferably seconds. Preferred dyes as initiators of choice for LWUV or visible light initiation are ethyl eosin, 2,2-dimethoxy-2-phenyl acetophenone, other acetophenone derivatives, and camphorquinone. In all cases, crosslinking and polymerization are initiated among macromers by a light-activated free-radical polymerization initiator such as 2,2-dimethoxy-2-phenylacetophenone or a combination of ethyl eosin (10^{-4} to 10^{-2} M) and triethanol amine (0.001 to 0.1 M), for example.

The choice of the photoinitiator is largely dependent on the photopolymerizable regions. For example, when the macromer includes at least one carbon-carbon double bond, light absorption by the dye causes the dye to assume a triplet state, the triplet state subsequently reacting with the amine to form a free radical which initiates polymerization. Preferred dyes for use with these materials include

eosin dye and initiators such as 2,2-dimethyl-2-phenylacetophenone, 2-methoxy-2-phenylacetophenone, and camphorquinone. Using such initiators, copolymers may be polymerized *in situ* by long wavelength ultraviolet light or by laser light of about 514 nm, for example.

Initiation of polymerization is accomplished by irradiation with light at a wavelength of between about 200-700 nm, most preferably in the long wavelength ultraviolet range or visible range, 320 nm or higher, most preferably about 514 nm or 365 nm.

There are several photooxidizable and photoreducible dyes that may be used to initiate polymerization. These include acridine dyes, for example, acriblarine; thiazine dyes, for example, thionine; xanthine dyes, for example, rose bengal; and phenazine dyes, for example, methylene blue. These are used with cocatalysts such as amines, for example, triethanolamine; sulphur compounds, for example, RSO_2R^1; heterocycles, for example, imidazole; enolates; organometallics; and other compounds, such as N-phenyl glycine. Other initiators include camphorquinones and acetophenone derivatives.

Thermal polymerization initiator systems may also be used. Such systems that are unstable at 37°C and would initiate free radical polymerization at physiological temperatures include, for example, potassium persulfate, with or without tetraamethyl ethylenediamine; benzoylperoxide, with or without triethanolamine; and ammonium persulfate with sodium bisulfite.

Applications for the Macromers.

Prevention of Surgical Adhesions.

A preferred application is a method of reducing formation of adhesions after a surgical procedure in a patient. The method includes coating damaged tissue surfaces in a patient with an aqueous solution of a

light-sensitive free-radical polymerization initiator and a macromer solution as described above. The coated tissue surfaces are exposed to light sufficient to polymerize the macromer. The light-sensitive free-radical polymerization initiator may be a single compound (e.g., 2,2-dimethoxy-2-phenyl acetophenone) or a combination of a dye and a cocatalyst (e.g., ethyl eosin and triethanol amine).

Controlled drug delivery.

A second preferred application concerns a method of locally applying a biologically active substance to tissue surfaces of a patient. The method includes the steps of mixing a biologically active substance with an aqueous solution including a light-sensitive free-radical polymerization initiator and a macromer as described above to form a coating mixture. Tissue surfaces are coated with the coating mixture and exposed to light sufficient to polymerize the macromer. The biologically active substance can be any of a variety of materials, including proteins, carbohydrates, nucleic acids, and inorganic and organic biologically active molecules. Specific examples include enzymes, antibiotics, antineoplastic agents, local anesthetics, hormones, antiangiogenic agents, antibodies, neurotransmitters, psychoactive drugs, drugs affecting reproductive organs, and oligonucleotides such as antisense oligonucleotides.

In a variation of the method for controlled drug delivery, the macromers are polymerized with the biologically active materials to form microspheres or nanoparticles containing the biologically active material. The macromer, photoinitiator, and agent to be encapsulated are mixed in an aqueous mixture. Particles of the mixture are formed using standard techniques, for example, by mixing in oil to form an emulsion, forming droplets in oil using a nozzle, or forming droplets in air using a nozzle. The

suspension or droplets are irradiated with a light suitable for photopolymerization of the macromer.

Tissue Adhesives.

Another use of the polymers is in a method for adhering tissue surfaces in a patient. The macromer is mixed with a photoinitiator or photoinitiator/cocatalyst mixture to form an aqueous mixture and the mixture is applied to a tissue surface to which tissue adhesion is desired. The tissue surface is contacted with the tissue with which adhesion is desired, forming a tissue junction. The tissue junction is then irradiated until the macromers are polymerized.

Tissue Coatings.

In a particularly preferred application of these macromers, an ultrathin coating is applied to the surface of a tissue, most preferably the lumen of a tissue such as a blood vessel. One use of such a coating is in the treatment or prevention of restenosis, abrupt reclosure, or vasospasm after vascular intervention. The photoinitiator is applied to the surface of the tissue, allowed to react, adsorb or bond to tissue, the unbound photoinitiator is removed by dilution or rinsing, and the macromer solution is applied and polymerized. As demonstrated below, this method is capable of creating uniform polymeric coating of between one and 500 microns in thickness, most preferably about twenty microns, which does not evoke thrombosis or localized inflammation.

Tissue Supports.

The macromers can also be used to create tissue supports by forming shaped articles within the body to serve a mechanical function. Such supports include, for example, sealants for bleeding organs, sealants for bone defects and space-fillers for vascular aneurisms. Further, such supports include strictures

to hold organs, vessels or tubes in a particular position for a controlled period of time.

The following examples are presented to describe preferred embodiments and utilities of the present invention and are not meant to limit the invention unless otherwise stated in the claims appended hereto. Taken together, the examples illustrate representative demonstrations of the best mode of implementing the invention as currently understood.

Table 1 shows the code names of the various macromers synthesized in or for use in the examples, along with their composition in terms of the molecular weight of the central PEG segment and the degree of polymerization of the degradable comonomer.

Table 1: Macromer Molecular Weight and Composition.

PEG molecular weight	Comonomer	D.P. of conomoner per OH group	Polymer Code
20,000	glycolide	15	20KG
18,500	glycolide	2.5	18.5K
10,000	glycolide	7	10KG
6,000	glycolide	5	6KG
4,000	glycolide	5	4KG
1,000	glycolide	2	1KG
20,000	DL-lactide	10	20KL
18,500	DL-lactide	10	18.5KL
10,000	DL-lactide	5	10KL
6,000	DL-lactide	5	6KL
1,000	DL-lactide	2	1KL
600	DL-lactide	2	0.6KL
600	DL-lactide + caprolactone(CL)	lactide 2; CL 1	0.6KLCL
18,500	caprolactone	2.5	18.5KCL
18,500	-	-	18.5KCO

Example 1: Synthesis of Photopolymerized Biodegradable Hydrogels.

PEG-based hydrogels

PEG-based biodegradable hydrogels are formed by the rapid laser or UV photopolymerization of water soluble macromers. Macromers, in turn, are synthesized by adding glycolic acid oligomers to the end groups of PEG and then capping with acrylic end groups. The PEG portions of the macromers confer water solubility properties, and subsequent polymerization results in cell-nonadhesive hydrogels. Glycolic acid oligomers serve as the hydrolyzable fraction of the polymer network, while acrylic end groups facilitate rapid polymerization and gelation of the macromers.

In preparation for synthesis, glycolide (DuPont) or DL-lactide (Aldrich) was freshly recrystallized from ethyl acetate. PEG oligomers of various molecular weight (Fluka or Polysciences) were dried under vacuum at 110°C prior to use. Acryloyl chloride (Aldrich) was used as received. All other chemicals were of reagent grade and used without further purification.

Macromer synthesis

A 250 ml round bottom flask was flame dried under repeated cycles of vacuum and dry argon. 20 gm of PEG (molecular weight 10,000), 150 ml of xylene and 10 μgm of stannous octoate were charged into the flask. The flask was heated to 60°C under argon to dissolve the PEG and cooled to room temperature. 1.16 gm of glycolide was added to the flask and the reaction mixture was refluxed for 16 hr. The copolymer was separated on cooling and was recovered by filtration. This copolymer was separated on cooling and recovered by filtration. This copolymer (10K PEG-glycolide) was used directly for subsequent reactions. Other polymers were similarly synthesized

using DL-lactide or ε-caprolactone in place of glycolide and using PEG of different molecular weights.

Synthesis of photosensitive oligomers (macromers):

19 gm of 10K PEG-glycolide copolymer was dissolved in 150 ml methylene chloride and refluxed with 1 ml acryloyl chloride and 1.2 ml of triethylamine for 12 hr under an argon atmosphere. The solid triethylamine hydrochloride was separated by filtration and the polymer was precipitated by adding the filtrate to a large excess of hexane. The polymer (capped by an acrylate at both ends) was further purified by repeated dissolution and precipitation in methylene chloride and hexane respectively.

Table 2 lists certain macromers synthesized. The degree of polymerization of the glycolide chain extender was kept low so that all polymers have approximately 10 ester groups per chain, or about 5 per chain end. When these polymers are photopolymerized, a crosslinked three-dimensional network is obtained. However, each chain segment in the resulting network needs just one ester bond cleaved at either end to "degrade." These ester cleavages enable the chain to dissolve in the surrounding physiological fluid and thereby be removed from the implant site. The resulting hydrolysis products, PEG and glycolic acid, are water soluble and have very low toxicity.

TABLE 2: Macromers Synthesized

Polymer Code	Mol. Wt. of Central PEG Chain (daltons)	%Glycolide in Extremeties	% ε-Caprolactone in Extremities	Calculated Mol. Wt. of Extremities (daltons)	Appearance
0.4K	400	100	-	580	Viscous liquid
1KG	1000	100	-	300	Viscous liquid
4KG	4000	100	-	232	White solid
10KG	10000	100	-	580	White solid
18.5KG	18500	100	-	1160	Yellow solid
col8.5KGCL	18500	50	-	580	White solid

Due to the presence of only a few units of glycolic acid per oligomeric chain, the solubility properties of the photocrosslinkable prepolymers are principally determined by the central PEG chain. Solubility of the macromers in water and methylene chloride, both of which are solvents for PEG, is not adversely affected as long as the central PEG segment has a molecular weight of 1,000 daltons or more. Solubility data for the prepolymers synthesized is given in Table 3.

Table 3: SOLUBILITY DATA

Solvent	1KG	4KG	10KG	18.5KG	TMP*
DMSO	–	■	–	■	■
Acetone	–	■	■	■	–
Methanol	–	■	–	■	–
Water	–	–	–	–	■
Hexane	■	■	■	■	■
Methylene Chloride	–	–	–	–	–
Cold Xylene	■	■	■	■	–
Hot Xylene	–	–	–	–	–
Benzene	■	■	■	■	–

– Soluble

■ Not Soluble

* Trimethylolpropane glycolide triacrylate

PEG chains with different degrees of polymerization of DL-lactide were synthesized to determine the degree of substitution for which water solubility of the macromers can be retained. The results are shown in Table 4. Beyond about 20% substitution of the hydrophilic PEG chain with hydrophobic DL-lactoyl or acrylate terminals leads to the macromers becoming insoluble in water, though they

are still soluble in organic solvents such as methylene chloride.

Table 4: Solubility of Macromers

D.P.* of Ethylene Oxide	D.P.* of lactide or glycolide	% extension of PEG chain	Solubility in water
420	4	0.1	soluble
420	10	2.4	soluble
420	20	4.8	soluble
420	40	9.5	soluble
420	80	19	insoluble
23	2	8.7	soluble
23	4	17.4	soluble
23	10	43.5	insoluble
23	40	174	insoluble
5	4	80	insoluble
10	4	40	soluble

* degree of polymerization

Photopolymerization

The macromers can be gelled by photopolymerization using free radical initiators, with the presence of two acrylic double bonds per chain leading to rapid gelation. A 23% w/w solution of various degradable polymers in HEPES buffered saline containing 3 μl of initiator solution (300 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone in n-vinyl pyrrolidone) was used. 100 μl of the solution was placed on a glass coverslip and irradiated with a low intensity long wavelength UV (LWUV) lamp (Blak-Ray, model 3-100A with flood). The times required for gelation to occur were noted and are given below. These times are typically in the range of 10 seconds. This is very significant because these reactions are carried out in air (UV initiated photopolymerizations are slow in air as compared to an inert atmosphere)

and using a portable, low powered long wave UV (LWUV) emitting source. Oxygen, which often inhibits free radical reactions by forming species which inhibit propagation, did not seem to slow down the polymerization. Such fast polymerizations are particularly useful in applications requiring *in situ* gelations. This rapid gelation is believed to be due to the formation of micelle-like structures between the relatively hydrophobic polymerizable groups on the macromer, thereby increasing the local concentration of the polymerizable species in aqueous solution and increasing polymerization rates.

Visible laser light is also useful for polymerization. Low intensity and short exposure times make visible laser light virtually harmless to living cells since the radiation is not strongly absorbed in the absence of the proper chromophore. Laser light can also be transported using fiber optics and can be focused to a very small area. Such light can be used for rapid polymerization in highly localized regions; gelation times for selected prepolymers are given in Table 5. In each case, 0.2 ml of a 23% w/v photosensitive oligomer solution is mixed with ethyl eosin (10^{-4} M) and triethanol amine (0.01 to 0.1 M) and the solution is irradiated with an argon ion laser (American argon ion laser model 905 emitting at 514 nm) at a power of 0.2-0.5 W/cm^2. The beam is expanded to a diameter of 3 mm and the sample is slowly scanned until gelation occurs.

Table 5: Gelation Times

Polymer	UV polymerization * gelation time (mean ±S.D.) (s)	Laser Polymerization** gelation time (s)
1KG	5.3±4.1	<1
4KG	14.7±0.5	<1
6KG	9.3±0.5	<1
10KG	18.±0.8	<1
10KL	7.7±0.5	<1
18KG	23.3±1.2	<1
20KG	13.3±0.5	<1

* Initiator: 2,2-dimethoxy-2-phenylacetophenone, concentration 900 ppm: 0.2 ml of 23% monomer solution in PBS

** Argon ion laser emitting at 514nm. power 3 W/cm^2: ethyloeosin, triethanol amine initiating system: 0.2 ml of 23% monomer solution in PBS

Biodegradability

Biodegradation of the resulting polymer network is an important criteria in many biomedical applications. Degradation of poly(glycolic acid and poly(DL-lactic acid) has been well documented in the literature. The degradation mainly takes place through the hydrolysis of the ester bond; the reaction is second order and highly pH dependent. The rate constant at pH 10 is 7 times faster than that at pH 7.2.

Such facile biodegradation is surprising because poly(α-hydroxyacidesters) are hydrophobic and highly insoluble in water. Accessibility of the polymer matrix to the aqueous surrounding is therefore limited. However, because the networks are hydrogels which are swollen with water, all the ester linkages in the network are in constant contact with water with the aqueous surroundings. This results in a uniform

bulk degradation rather than a surface degradation of these gels.

Table 6 gives hydrolysis data for some of these networks; times listed are for complete dissolution of 60 mg of gel at pH 7.2 and 9.6. As noted, most of the gels dissolve within 12 hours at pH 9.6. 18.5k gel dissolves within 2.5 hr at pH 9.6 whereas 18.5KCO gel does not dissolve in 3 days, indicating that the lactoyl, glycoloyl, or ϵ-caprolactoyl ester moiety is responsible for degradation of these networks. It also can be seen that the 18.5KG gel hydrolyzes more rapidly than the 4KG gel. This may be due to the reduced hydrophilicity and higher crosslink density of the latter gel.

Table 6: Hydrolysis Data

Oligomer used for gelation	Time taken to dissolve gel at pH 9.6 (h)	Time taken to dissolve gel at pH 7.2 (days)
4KG	6.2	5.5
10KG	12.25	5.5
18.5KG	2.25	>7
18.5KCL	>5 days	>7
18.5KCO	>5 days	>7

Characterization of macromers

FTIR spectra of the prepolymers were recorded on a DIGILAB model FTS 15/90. The absorption at 1110 cm^{-1} (characteristic C-0-C absorption of PEG) shows the presence of PEG segments. The strong 1760 cm^{-1} absorption shows the presence of glycolic ester. The absence of hydroxyl group absorption around 3400 cm^{-1} and a weak acrylic double bond absorption at 1590 cm^{-1} shows the presence of acrylic double bonds at the end groups.

500 MHz proton and 125 MHz carbon-13 spectra were recorded on a GE 500 instrument. The presence of a very strong peak at 4.9 ppm due to CH_2 methylene from the PEG segment, a peak at 5.09 ppm due to the

glycolic ester segment and an acrylic proton singlet at 5.8 ppm can be easily seen from proton NMR. The estimated molecular weight of PEG segment and glycolic acid segment for different copolymers is shown in Table 2. The carbonyl peak at 169.39 ppm from glycolic acid and 36.5 ppm peak from methylene carbons from PEG in carbon-13 NMR are consistent with the reported chemical composition of these copolymers.

Differential scanning calorimetry (Perkin Elmer DSC-7) was used to characterize the oligomers for thermal transitions. The oligomers were heated from -40°C to 200°C at a rate of 20°C/min, presumably causing polymerization. The polymer was then cooled to -40°C at a rate of 60°C/min and again heated to 200°C at a rate of 20°C/min. The first scans of biodegradable 18.5K PEG glycolide tetraacrylate (18.5KG) oligomer were compared to that of the non-degradable 18.5K PEG tetraacrylate (18.5KCO) scan. It was seen that a glass transition appears in the 18.5KG at -2°C while no such transition exists in the 18.5KCO. A small melting peak at 140°C was also evident due to the few glycolic acid mers which can crystallize to a limited extent. The melting peak for PEG is shifted downwards in 18.5KG to 57°C from 60.7°C for 18.5KCO. This is probably due to disturbance of the PEO crystalline structure due to the presence of the glycolic acid linkages. In the third cycle, by which time the oligomers have presumably polymerized, the Tg and Tm transitions for the glycolide segments can no longer be seen, indicating that a crosslinked network has formed and the glycolic acid segments are no longer capable of mobility.

The degree of polymerization (D.P.) of the degradable segments added to the central water soluble PEG chain was determined in several cases using ^1H NMR. The experimentally determined D.P. was seen to be in good agreement with the calculated number, as shown by

Figure 1A. Thus, the ring opening reaction initiated by the PEG hydroxyls proceeds to completion, giving quantitative yields.

Determination of Total Water, Free Water Bound Water

Solutions of various degradable macromers were made as described above. Gels in the shape of discs were made using a mold. 400 μl of solution was used for each disc. The solutions were irradiated for 2 minutes to ensure thorough gelation. The disc shaped gels were removed and dried under vacuum at 60°C for 2 days. The discs were weighed (W1) and then extracted repeatedly with chloroform for 1 day. The discs were dried again and weighed (W2). The gel fraction was calculated as W2/W1. This data appears in Table 7.

Subsequent to extraction, the discs were allowed to equilibrate with PBS for 6 hours and weighed (W3 after excess water had been carefully swabbed away). The total water content was calculated as (W3-W2) X 100/W3. Differential scanning calorimetry (DSC) was used to determine the amount of free water that was available in the gels. A scan rate of 20°C/min was used and the heat capacity for the endotherm for water melting was measured (H1). The heat capacity of HBS was also measured (H2). The fraction of free water was calculated as H1/H2. The residual water was assumed to be bound due to hydrogen bonding with the PEO segments. The presence of free water in the gels was indicated. This free water can be expected to help proteins and enzymes entrapped in such gels in maintaining their native conformation and reducing deactivation. Thus these gels would appear to be suited for controlled release of biological micromolecules. The data for gel water content is summarized in Table 7

Table 7: Hydrogel Water content

Polymer Code	% Free Water	% Bound Water	% Total Water	% Gel Content
1KG	68.4	14	82.3±2.6	61.3±5.2
4KG	78.0	9.3	87.3±1.8	56.3±0.9
6KG	74.8	13.4	88.1±3.3	66.5±2.35
10KG	83.7	10.8	94.5±0.5	54.3±0.6
10KL	82.0	9.7	91.7±0.5	63.9±3.7
18.5KG	71.8	22.3	94.0±0.4	47.0±4.9
20KG	79.8	14.8	94.5±0.4	44.5±4.8

Example 2: Use of multifunctional macromers.

30 g of a tetrafunctional water soluble PEG (MW 18,500) (PEG 18.5k) was dried by dissolving the polymer in benzene and distilling off the water benzene azeotrope. In a glove bag, 20 g of PEG 18.5 k, 1.881 g of glycolide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 h. at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 3: Synthesis of a photosensitive macromer containing DL-lactide.

PEG (MW) 20,000) (PEG 20k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 32.43 g of PEG 20k, 2.335 g of DL-lactide and 15 mg of stannous octoate were charged into a 100 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by pouring into an excess of dry ethyl ether. It was redissolved in 200 ml of dichloromethane in a 500 ml round bottom flask cooled to 0°C. To this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day.

Example 4: Synthesis of a Photosensitive Precursor Containing DL-Lactide and ϵ-Caprolactone.

PEG (MW 600) (PEG 0.6k) was dried by dissolving in benzene and distilling off the water benzene azeotrope. In a glove bag, 0.973 g of PEG 0.6k, 0.467 g of DL-lactide along with 0.185 g of ϵ-caprolactone and 15 mg of stannous octoate were charged into a 50 ml round bottom flask. The flask was capped with a vacuum stopcock, placed into a silicone oil bath and connected to a vacuum line. The temperature of the bath was raised to 200°C. The reaction was carried out for 4 hours at 200°C and 2 hours at 160°C. The reaction mixture was cooled, dissolved in dichloromethane and the copolymer was precipitated by

pouring into an excess of dry ethyl ether. It was redissolved in 50 ml of dichloromethane in a 250 ml round bottom flask cooled to 0°C. to this flask, 0.854 g of triethylamine and 0.514 ml of acryloyl chloride were added under nitrogen atmosphere and the reaction mixture was stirred for 12 hours at 0°C. The triethyl amine hydrochloride was separated by filtration and the copolymer was recovered from filtrate by precipitating in diethyl ether. The polymer was dried at 50°C under vacuum for 1 day and was a liquid at room temperature.

Example 5: Selection of dyes for use in photopolymerization.

It is possible to initiate photopolymerization with a wide variety of dyes as initiators and a number of electron donors as effective cocatalysts. Table 8 illustrates photopolymerization initiated by several other dyes which have chromophores absorbing at widely different wavelengths. All gelations were carried out using a 23% w/w solution of 18.5KG in HEPES buffered saline. These initiating systems compare favorably with conventional thermal initiating systems, as can also be seen from Table 8. Other photoinitiators that may be particularly useful are 2-methoxy-2-phenyl acetophenone and camphorquinone.

Table 8: Polymerization Initiation of 18.5KG PEG

INITIATOR	LIGHT SOURCE*	TEMPERATURE °C	GEL TIME (SEC)
Eosin Y, 0.00015M; Triethanolamine 0.65M	S1 with UV filter	25	10
Eosin Y, 0.00015M; Triethanolamine 0.65M	S4	25	0.1
Methylene Blue, 0.00024M; p-toluenesulfinic acid, 0.0048M	S3	25	120
2,2-dimethoxy-2-phenyl acetophenone 900 ppm	S2	25	8
Potassium persulfate 0.0168M	-	75	180
Potassium Persulfate 0.0168M; tetramethyl ethylene-diamine 0.039M	-	25	120
Tetramethyl ethylene-diamine 0.039M; Riboflavin 0.00047M	S1 with UV filter	25	300

***LIST OF LIGHT SOURCES USED**

CODE SOURCE

S1 Mercury lamp, LEITZ WETSLER Type 307-148.002, 100W

S2 Black Ray longwave UV lamp, model B-100A W/FLOOD

S3 MELLES GRIOT He-Ne laser, 10mW output, 1=632 nm

S4 American laser corporation, argon ion laser, model 909BP-15-01001; λ=488 and 514 nm

Numerous other dyes can be used for photopolymerization. These dyes include but are not limited to: Erythrosin, phloxine, rose bengal, thioneine, camphorquinone, ethyl eosin, eosin, methylene blue, and riboflavin. The several possible cocatalysts that can be used include but are not limited to: N-methyl diethanolamine, N,N-dimethyl benzylamine, triethanol amine, triethylamine, dibenzyl

amine, N-benzyl ethanolamine, N-isopropyl benzylamine, and N-vinyl pyrrolidinone.

Example 6: Thermosensitive Biodegradable Gels from N-Isopropyl Acrylamide.

Synthesis of low molecular weight polyisopropyl acrylamide.

N-isopropyl acrylamide (NIPAAm) was recrystallized from 65:35 hexane benzene mixture. Azobisisobutyronitrile (AIBN) was recrystallized from methanol. 1.5 g of NIPAAm was polymerized using 3 mg of AIBN and 150 mg of mercaptoethanol in 1:1 acetone water mixture (24 hours at 65°C). The viscous liquid after polymerization was purified by dissolving in acetone and precipitating in diethyl ether. Yield 80%.

This hydroxy terminated low molecular weight poly(NIPAAm) was used in chain extension reactions using glycolide and subsequent endcapping reaction using acryloyl chloride as described in other examples.

1 g of modified poly(NIPAAm) based oligomer and 0.2 g 1KL were dissolved in water at 0°C and polymerized at 0°C using 2-2-dimethoxy-2-phenylacetophenone (900 PPM).

Example 7: *In Vitro* Degradation

The gels were extracted as described in Example 1 to remove the unpolymerized macromer fraction fraction and the gels were then placed in 50 mM HEPES buffered saline (0.9% NaCl), pH 7.4 at 37°C. Duplicate samples were periodically removed, washed with fresh HBS and dried at 100°C for 1 day and weighed to determine mass loss in the gel. The compositions of the various gels used were the same as described in the previous examples. Table 9 shows the extent of degradation of these gels given as percent of mass lost over time. The respective times are given in parenthesis along with the mass loss data.

Table 9: Gel Degradation

1KG	20.1% (1 d), 20.36±0.6 (2d), 21.7± (6d), 28.8±16.6 (10 d) estimated total Degradation time 45 days.
4KG	38.9 (1d), 60.3±4.2 (2d), 78.9 (3d), 99.3±4.7 (6d). Total degradation time 5.5 days.
6KG	18.3±6.8 (1d), 27.4±1.0 (2d), 32.8±11.3 (3d), 104.8±3.2 (5d). total degradation time 4.5 days 10KG 0.6±0.6 (8 hr), 100 (1d). Total degradation time 1 day.
10KL	10.0±4.84 (2d), 6.8±1.7 (3d), 4.5±3.1 (6d), 8.0±0.2 (10d). Total degradation time estimated to be 20 days.
20KG	68.1±4.2 (8hr), 99.7±0.3 (1d). Total degradation time 15 hr.

Example 8: Fibroblast adhesion and spreading.

The *in vitro* response of Human foreskin fibroblast (HFF) cells to photopolymerized gels was evaluated through cell culture on polymer networks. 0.2 ml of monomer solution was UV polymerized on an 18 x 18 mm glass coverslips under sterile conditions. HFF cells were seeded on these gels at a cell density of 1.8 x 10^4 cells/sq cm of coverslip area in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum. The gels were incubated for 6 hr at 37°C in a 5% CO_2 environment, at the end of which they were washed twice with phosphate buffered saline (PBS). The adherent cells were fixed using a 2% glutaraldehyde solution in PBS. The gels were examined under a phase contrast microscope at a magnification of 200X, and the number of adherent and spread cells evaluated by examining five fields selected at predetermined locations on the coverslips.

The number of adherent cells is reported in Table 10 along with those for glass control surfaces.

Cell adhesion is seen to be dramatically lowered on gel-coated glass.

Table 10: Cell Adhesion

Surface	Attached Cells/cm^2
glass	13220±3730
18.5KG	250±240
18.5KCL	1170±1020
18.5KCO	390±150

Typical photographs of these cells on the 18.5KCL gel surfaces and on control glass surfaces are shown in Figures 2A and 2B. It can be easily seen from Table 10 that these gels are highly resistant to cellular growth. Even the 18.5KCL is still less than 10% of the glass. Cells attached to the glass surface show a flattened and well-spread morphology whereas the few cells that are attached to the gel are rounded and loosely attached. This may result from the fact that hydrated PEG chains have a high motility and have been shown to be effective in minimizing protein adsorption. One of the mechanisms by which cell adhesion is mediated is through the interaction of cell surface receptors with adsorbed cell adhesion proteins. Thus the reduction in overall protein adsorption results in minimal cell adhesion protein adsorption and reduced cell adhesion.

Example 9: Release of Protein (Bovine Serum Albumin) from Polymers.

1KG was used for this study. This macromer was liquid at room temperature and was used as such. 1 mg of bovine serum albumin (BSA) was added per ml of monomer solution along with 0.9 mg/ml of 2,2-dimethoxy-2-phenyl-acetophenone as initiator. The protein was dissolved in the monomer solution and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 min. Two such discs were placed in a flask containing 20 ml of PBS and incubated at 37°C. Two aliquots of 20 μl each were removed from

these flasks periodically and the amount of BSA released was assayed using the Bio-Rad total protein assay. The release profile for BSA is shown in Figure 3A. It can be seen that the release of BSA is relatively steady over more than a month.

Example 10: Enzyme Release Assay

Water solubility of the macromers means gelation can be carried out in a non-toxic environment. This makes these materials suitable for intraoperative uses where *in situ* gelation is needed. Since the precursors are water soluble, the gels can be used as drug delivery vehicles for water soluble drugs, especially macromolecular drugs such as enzymes, which would otherwise be denatured and lose their activity. Release of lysosome and tPA from the polymers was used to illustrate the feasibility of using biodegradable hydrogels for controlled release of biomolecules.

Lysozyme release

The enzyme lysozyme (MW:14,400) is a convenient model for release of a low molecular weight protein from a biodegradable gel. The Biorad total protein assay was used to quantify the enzyme released. The enzyme was dissolved in PBS at a concentration of 20 mg/ml. The monomer PEG-dl-lactic acid-diacrylate was dissolved in PBS to produce a 40% solution. The lysozyme solution was added to the monomer solution to attain a 24% monomer solution. The monomer/lysozyme solution was polymerized under UV in a cylindrical mold, using 30 μl of the initiator 2,2-dimethoxy-2-phenyl-acetophenone in 1-vinyl-2-pyrrolidone (30 mg/ml) as the initiator. The polymer was cut into 10 equal sized pieces and immersed in 10 ml PBS. Samples of the PBS were withdrawn at intervals and assayed for lysozyme released into the PBS. Lysozyme was released from the PEG-DL-lactic acid-diacrylate gel over an 8 day interval, with the maximum rate of release

occurring within the first 2 days, as shown by Figure 3B.

Release of recombinant t-PA

Three macromers were used for these studies: 1KL, 4KG, and 18.5KG. The 1KL macromer was liquid at room temperature and was used as such. The second macromer, 4KG, was used as a 75% w/w solution in PBS. The third composition was a mixture of equal parts of 1KL and a 50% w/w solution of 18.5KG. 3.37 mg of tissue plasminogen activator (single chain, recombinant, M.W. 71,000) was added per gram of macromer solution along with 0.9 mg/ml of 2,2 dimethoxy 2 phenyl acetophenone as initiator. The protein was dissolved with the macromer and disc shaped gels were made by exposing 0.2 g of macromer mixture to LWUV for 1 minute. Two such discs were rinsed with PBS, placed in a flask containing 5 ml of PBS and incubated at 37°C. Two aliquots of 100 μl each were removed from these flasks periodically and the amount of active t-PA released was assayed using a chromogenic substrate assay (Kabi-vitrum). The release profiles from the 1K lactide gels, 4K glycolide gels, and the 50/50 1K glycolide/18.5K glycolide are shown in Figures 4A - 4C. Fully active tPA can be released for periods up to at least two months.

By selecting an appropriate formulation, the release rate can be tailored for a particular application. It is also possible to combine formulations with different molecular weights so as to synergistically achieve appropriate attributes in release and mechanical characteristics.

For prevention of postoperative adhesions, in addition to the barrier effect of the gels, the gels can be loaded with a fibrinolytic agent to lyse incipient filmy adhesions which escape the barrier

effect. This further enhances the efficacy of biodegradable gels in adhesion prevention.

Example 11: Toxicity of Polymers and Commercial Adhesives.

To evaluate the toxicity of *in situ* polymerization of the macromer solutions described herein, as compared to commercial adhesives, 100 μl of 18.5KCO prepolymer solution was placed on the right lobe of a rat liver and gelled by exposing it to LWUV for 15 sec; similarly, a few drops of a n-butyl cyanoacrylate based glue were placed on the left lobe. The liver was excised after a week, fixed in 10% neutral buffered formalin, blocked in paraffin, sectioned and stained using hematoxylin and eosin.

No adverse tissue reaction was evident on the surface of the lobe exposed to the biodegradable gel. No inflammatory reaction to the polymerization process can be seen. The epithelium looks normal, with no foreign body reaction.

In comparison, the lobe exposed to cyanoacrylate glue shows extensive tissue necrosis and scarring with 10-30 cell deep necrotic tissue. Fibrosis is evident in the necrotic portions close to underlying normal tissue.

Example 12: Prevention of Post-Surgical Adhesions with Photopolymerized Biodegradable Polymer.

A viscous sterile 23% solution in phosphate buffered saline (8.0 g/l NaCl, 0.201 g/l KCl, 0.611 g/l Na_2HPO_4, 0.191 g/l KH_2PO_4, pH 7.4) of polyethylene glycol (M.W. 18,500) which has been chain extended on both ends with a short polyglycolide repeat unit (average number of glycolidyl residues: 10 on each end) and which has been subsequently terminated with an acrylate group was prepared. Initiator needed for the crosslinking reaction, 2,2-dimethoxy-2-phenyl acetophenone, was added to the macromer solution to

achieve an initiator concentration of 900 ppm. A 30 second exposure to a long wave UV lamp (Blak Ray) is sufficient to cause polymerization.

Animal models evaluated

Animal models evaluated included a rat cecum model and a rabbit uterine horm model. In the rat cecum mode, 6 out of 7 animals treated with the macromer solution showed no adhesions whatsoever, while untreated animals showed consistent dense adhesion formation. In the rabbit uterine horn model, a significant ($p<0.01$) reduction in adhesion formation was seen in the animals treated with the gel. Studies conducted in rats using only the ungelled viscous precursor solution (no LWUV) failed to prevent the formation of adhesions.

Rat cecum model

Twenty-one Sprague Dawley male rats having an average weight of 250 gm were divided into three groups for treatment and two for controls. The abdomen was shaved and prepared with a betadine solution. A midline incision was made under Equithesin anesthesia. The cecum was located and 4 to 5 scrapes were made on a region about 2 x 1 cm on one side of the cecum, using a 4 x 4 in gauze pad to produce serosal injury and punctate bleeding. The abdominal incisions in these animals were closed using a continuous 4-0 silk suture for the musculoperitoneal layer and 7.5 mm stainless steel staples for the cutaneous layer. A topical antibiotic was applied at the incision site.

The first group consisted of 7 animals serving as controls without treatment, to confirm the validity of the model. The second group served as a control with the application of the precursor but without photopolymerization to form the hydrogel. After induction of the cecal injury, about 0.25 ml of the precursor solution was applied to the injury site

using a pipet. The abdominal incision was then closed as above.

The third group served as the gel treatment group and was prepared as the second group except that the precursor film was exposed to a LWUV lamp for 45 seconds to cause gelation. Both the obverse and reverse sides of the cecum were similarly treated with precursor and light. No attempt was made to dry the surface of the tissue, to remove blood, or to irrigate the area prior to treatment.

The animals were sacrificed at the end of two weeks by CO_2 asphyxiation. The incisions were reopened and adhesions were scored for location, extent, and tenacity. The extent of adhesions was reported as a percentage of the traumatized area of the cecum which forms adhesions with adnexal organs or the peritoneal wall. Tenacity of the adhesions was scored on a scale from 0 to 4: no adhesions - grade 0; tentative transparent adhesions which frequently separate on their own - grade 1; adhesions that give some resistance but can be separated by hand - grade 2; adhesions that require blunt instrument dissection to separate - grade 3; and dense thick adhesions which require sharp instrument dissection in the plane of the adhesion to separate -grade 4.

Rat cecum model results

The control group without treatment shows consistently dense and extensive adhesions. The extent of abraded area covered with adhesions was seen to be 73±21% (mean ± S.D., n=7). The severity of adhesions was grade 3.5±0.4. Most of the adhesions were dense and fibrous, involving the cecum with itself, with the peritoneal wall and with other organs such as the liver, small intestine, and large intestine. Frequently the nesentery was seen to be involved in adhesions. In the control group with the application of precursor solution but without gelation

by exposure to the LWUV lamp, the extent of adhesion was 60 ± 24% (n=7), and the severity of adhesions was 3.1 ± 0.4. In the gel treated group, the cecum was seen to be completely free of adhesions in 6 out of 7 animals. In one case, a grade 2 adhesion was seen with the mesentery over 10% of the area and a grade 2.5 adhesion was seen over 15% of the area, bridging the cecum to the sutures on the site of the incision in the peritoneal wall. The overall adhesion extent for the group was 4%, and the overall severity was 0.32. No evidence of residual gel was visible, the gel presumably having degraded within the prior two weeks. The cecum appeared whitish with a fibrous layer on the surface in the control group, but the tissue appeared healthy and normal in animals treated with the gel.

Rabbit uterine horn model

Eight sexually mature female New Zealand rabbits between 2 and 3 kg in weight were prepared for surgery. A midline incision was made in the lower abdominal region under Rompun, Ketamine, and Acepromazine anesthesia. The uterine horns were located and the vasculature to both horns was systematically cauterized to induce an ischemic injury. One animal was rejected from the study due to immature uterine horns. Seven rabbits were selected for the treatment with only the photopolymerizable hydrogel and two animals were selected for evaluating the combined efficacy of the hydrogel with a fibrinolytic agent, tissue plasminogen activator (tPA). 5 mg of tPA/ml macromer solution was used in the latter case. After cauterization, macromer solutions (0.5 ml) were applied along the horn and allowed to coat the surface where the cauterization injury had been induced. After uniform application of the solution was complete, the horns were exposed to a LWUV lamp for 1 min to induce gelation. The procedure

was repeated on the reverse side of the horns. The incisions were then closed using a continuous 2-0 Vicryl (Ethicon) suture for the musculoperitoneal layer and a 0 Vicryl (Ethicon) suture for the cutaneous layer. No prophylactic antibiotics were administered. No postoperative complications or infections were observed. Five animals were used in the control group. The ischemic injury was made as described and the incision was closed without the application of the precursor; all techniques were identical between the treatment group and the control group.

Controls were used where the same animal model was subjected to surgery without application of the macromer; all surgical techniques were identical between the treatment group and the historical controls.

The rabbits were reoperated under Ketamine anesthesia at the end of two weeks to evaluate adhesion formation; they were sacrificed by introcardiac KCl injection. Adhesion formation was evaluated for extent and tenacity. Extent of adhesion formation was evaluated by measuring the length of the uterine horn that formed adhesions with itself or with the peritoneal wall or other organs. Tenacity of adhesion was classified as either filmy or fibrous. Filmy adhesions were usually transparent, less strong, and could be freed by hand. The fibrous adhesions were dense, whitish, and usually required sharp instrument dissection to be freed. In cases where only a single filmy adhesion band was evident, a score of 5% was assigned.

Typical samples of the horn were excised for histology and were fixed in a 10% neutral buffered formalin solution. Paraffin sections of the samples were stained using hematoxylin and eosin.

Rabbit uterine horn model results

The adhesion score is the % of affected area occupied by the adhesions, with grading of each as being filmy or fibrous. Distorted horn anatomies were observed in control animals. The mean score in the control group was 50 ± 15% of the affected area of the horn being occupied by adhesions with 10% of these being filmy and 90% fibrous. Distorted horn anatomies were observed, as can be seen from Figure 5A which presents a superior view of the uterine horn in an animal used as a control, which showed adhesions over 66% of the horn surface. The group of animals treated only with the photopolymerized macromer showed an adhesion score of 13±11.4% (n=10). Of these, 4 animals showed less than 5% adhesions with only an occasional filmy band visible.

The animals treated with photopolymerized gel containing tPA showed further improved results over the "gel only" animals. One animals showed a filmy band on both the right and left horn. They were assigned a score of 5% with a total score of 10%. The other animal did not show any adhesions at all. Thus the total score for these animals was 5±5%.

Figure 5B shows normal horn anatomy in a typical horn which has undergone gel treatment. Adhesions are filmy in all cases and no dense bands are seen. No traces of the remaining gel could be observed. Typical samples of horns showing filmy adhesions showed some fibrous tissue with a 6-15 cell thick layer of fibroblasts showing some collagen fibrils but no formation of dense collagen fibers. The horns showing no adhesions occasionally showed a 1-4 cell thick layer of fibroblasts, but mostly a normal epithelium with no evidence of inflammatory cells.

This same procedure was slightly modified as described below as a better mode of using the polymers

to prevent postoperative adhesions using the rat uterine horn model.

Female rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally), and a midline laparotomy was performed. The uterine horns were exposed, and the vasculature in the arcade feeding the horns was systematically cauterized using bipolar cautery; the most proximal and most distal large vessel on each horn were not cauterized. Following this, the antimesenteric surface of each horn was cauterized at two 1 mm diameter spots on each horn, each separated by a 2 cm distance, the pair centered along the length of each horn. Following injury, 0.5 ml of macromer solution was applied per horn and was gelled by exposure to long wavelength ultraviolet light (365 nm, approximately 20 mW/cm^2) for 15 sec per surface on the front side and on the back side each. The uterus was replaced in the peritoneal cavity, and the musculoperitoneal and skin layers were closed.

The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. In one batch, Batch A, the degree of acrylation was determined by NMR to be approximately 75%, and in another, Batch B, it was determined to be greater than approximately 95%. The macromer was dissolved in saline at a specified concentration, and the initiation system used was 2,2-dimethoxy-2-phenyl acetophenone from a stock solution in N-vinyl pyrrolidinone, the final concentration of 2,2-dimethoxy-2-phenyl acetophenone being 900 ppm and the final concentration of N-vinyl pyrrolidinone being 0.15%.

In one set of experiments, macromer from Batch A was applied in varying concentrations, and adhesions

were scored at 7 days postoperatively. Scoring was performed by two means. The length of the horns involved in adhesions was measured with a ruler, and the fraction of the total length was calculated. The nature of the adhesions was also scored on a subjective scale, 0 being no adhesions, 1 being filmy adhesions that are easily separated by hand, and 2 being dense adhesions that can only be separated by sharp instrument dissection. Furthermore, one of the samples contained tissue-plasminogen activator (t-PA), which is known to reduce adhesions, at a concentration of 0.5 mg/ml (0.5%) macromer solution. The results are shown in Table 11 for macromer batch A and batch B.

In a third set of experiments, adhesions were formed in female rats as described above, and the adhesions were surgically lysed 7 days after the initial surgery. The extent and grade of adhesions was scored during lysis. The animals were divided into two groups, and one group was treated with macromer from Batch B at a concentration of 10%. The results are shown in Table 11 as batch B, 10%.

Table 11: Reduction of Adhesions with Polymer.

Concentration macromer	Extent of adhesions % (S.D.)	Grade of adhesions (0-2)	Number of Animals
Polymer A			
15%	24.6 (3.1)	1.1 (0.1)	7
20%	33.6 (9.8)	1.2 (0.3)	7
25%	37.5 (11.1)	1.2 (0.1)	7
30%	54.2 (12.0)	1.6 (0.4)	6
20% + t-PA	18.3 (6.4)	1.1 (0.1)	6
Control (saline)	72.6 (18.7)	1.5 (0.2)	7
Polymer B			
5%	22.1 (4.2)	1.2 (0.1)	7
10%	10.0 (5.1)	1.0(0)	7
15%	17.8 (5.7)	1.0 (0)	7
20%	26.3 (11.4)	1.4 (0.2)	7
Control (saline)	75.9 (4.4)	1.8 (0.3)	7
Polymer B, 10%			
Scoring performed at:	group that became:		
time of lysis	Controls	85.9 (9.7)	1.8 (0.1)	7
Time of lysis	Treatment	79.4 (6.8)	1.7 (0.2)	7
7 days post-lysis	Controls	78.8 (11.3)	1.8 (0.1)	7
7 days post-lysis	Treatment	28.2 (5.1)	1.0 (0)	7

The above results illustrate that the photopolymerized macromer can reduce or prevent post operative adhesions in both primary adhesions and adhesiolysis models, and moreover that the gel can be used to locally release a drug to exert a combined beneficial effect.

Example 13: Nerve anastomosis.

The sciatic nerve of a rat was aseptically severed using a scalpel and allowed to pull apart. The two ends of the nerve were reopposed using sterile forceps, and a 50% solution in buffer of polymer 1KL,

a macromer made from PEG 1K with lactide chain extension and acrylate termination, with 0.1% 2,2-dimethoxy-2-phenoxy acetophenone was applied to the nerve stumps. The affected area was illuminated with a 100 W LWUV lamp for 60 seconds, and an adhesive bond was observed to form between the proximal and distal nerve stumps.

To ensure the biocompatibility of the applied material with the nerve tissue, the same solution of macromer was applied to nonsevered rat sciatic nerves, and the area of the incision was closed using standard small animal surgical technique. The area was reopened at 1 hour or 24 hour postoperatively, and the affected area of the nerve was removed en block and prepared for transmission electron microscopy. No morphological differences were observable between the treated nerves at either time point as compared to control rat sciatic nerves that were otherwise nonmanipulated, even though they had been traumatized and manipulated.

Example 14: Evaluation of PEG Based Degradable Gels as Tissue Adhesives.

Abdominal muscle flaps from female New Zealand white rabbits were excised and cut into strips 1 cm X 5 cm. The flaps were approximately 0.5 to 0.8 cm thick. A lap joint, 1 cm X 1 cm, was made using two such flaps. Two different compositions, 0.6KL and 1 KL, were evaluated on these tissues. Both these compositions were viscous liquids and were used without further dilution. 125 μl of ethyl eosin solution in N-vinyl pyrrolidone (20 mg/ml) along with 50 μl of triethanolamine was added to each ml of the adhesive solution. 100 μl of adhesive solution was applied to each of the overlapping flaps. The lap joint was then irradiated by scanning with a 2 W argon ion laser for 30 sec from each side. The strength of the resulting joints was evaluated by measuring the

force required to shear the lap joint. One end of the lap joint was clamped and an increasing load was applied to the other end, while holding the joint was clamped and an increasing load was applied to the other end, while holding the joint horizontally until it failed. Four joints were tested for each composition. The 1KL joints had a strength of 6.6±1.0 KPa (mean ±S.D.), while the 0.6KL joints had a strength of 11.4±2.9KPa. It is significant to note that it was possible to achieve photopolymerization and reasonable joint strength despite the 6-8 mm thickness of tissue. A spectrophotometric estimate using 514 nm light showed less than 1% transmission through such muscle tissue.

Example 15: Coupling of Photopolymerizable Groups to Proteins (Albumin).

PEG (M.W. 2,000) monoacrylate (5g) was dissolved in 20 ml dichloromethane. Triethyl amine (0.523 g) and 2,2,2-trifluoroethanesulfonyl chloride (tresyl chloride) (0.017 g) were added and the reaction was allowed to proceed for 3 hours at 0°C under nitrogen atmosphere. The reaction mixture was then filtered and the dichloromethane evaporated to dryness. The residue was redissolved in a small amount of dichloromethane and precipitated in diethyl ether. The polymer was then filtered and dried under vacuum for 10 hours and used directly in the subsequent reaction with albumin.

1 g of bovine serum albumin was dissolved in 200 ml of sodium bicarbonate buffer at pH 9. Tresyl activated PEG monoacrylate (5 g) was added and the reaction was stirred for 24 hours at 25°C. Albumin was separated by pouring the reaction mixture into acetone. It was further purified by dialysis using a 15,000 daltons cutoff dialysis membrane. A 10% w/v solution of the PEG acrylated albumin could be photopolymerized with long wave UV radiation using 0.9

mg/ml of 2,2 dimethoxy 2 phenylacetophenone as the initiator. In this gel the degradable segment is the protein albumin.

Example 16: Modification of Polysaccharides (Hyaluronic Acid)

In a dry 250 ml round bottom flask, 10 grams of PEG 400 monomethacrylate was dissolved in 100 ml dry dioxane, to which 4.053 g of carbonyl diimidazole (CDI) was slowly introduced under nitrogen atmosphere and the flask was heated to 50°C for 6 h. Thereafter the solvent was evaporated under vacuum and the CDI activated PEG monomer was purified by dissolving in dichloromethane and precipitating in ether twice.

1 g of hyaluronic acid, 5 g of CDI activated PEG 400 monoacrylate were dissolved in 200 ml sodium borate buffer (pH 8.5) and the solution was stirred for 24 hours. It was then dialyzed using a 15,000 dalton cutoff dialysis membrane to remove unreacted PEG. A 10% w/v solution of the acrylated hyaluronic acid was photopolymerized with long wave UV radiation, using 0.9 mg/ml of 2,2-dimethoxy-2-phenylacetophenone as the initiator. In this gel, the degradable region is hyaluronic acid.

Example 17: PEG Chain Extended with Polyorthocarbonates and Capped with Urethane Methacrylate.

3, 9-bis(methylene) 2,4,8,10-tetraoxaspiro [5,5] undecane (1g) and polyethylene glycol (molecular weight, 1,000, 7.059 g) were weighed into a 250 ml Schlenk tube under dry nitrogen atmosphere in a glove bag. 50 ml of dry tetrahydrofuran was introduced under nitrogen atmosphere and reaction mixture was stirred for 6 hours at 50°C. This is a typical step growth reaction with a disturbed stoichiometry, resulting in low molecular weight poloyorthocarbonate with terminal hydroxy groups. The oligomer was separated by precipitating in hexane and dried under

vacuum. 5 g of oligomer was redissolved in dry THF to which 20 μl of dibutyltindilaurate and 2 ml of 2-isocyanatoethyl methacrylate were slowly introduced and temperature was raised to 50°C. It was held there for 6 hours and cooled. The product was separated by precipitation in hexane. In this gel, the degradable region is a polyorthocarbonate.

Example 18: Microencapsulation of Animal Cells.

A 23% w/w solution of 18.5KG in HEPES buffered saline (5 ml) was used to resuspend 10^6 CEM-SS cells. Ethyl eosin (10^{-4} M) was used as a solution in N-vinyl pyrrolidone as the initiator and triethanolamine (0.01 M) was used as the coinitiator. The solution was then exposed through a coextrusion apparatus to an argon ion laser (514 nm, 2 Watts). The coextrusion apparatus had mineral oil as the fluid flowing annularly (flow rate 4 ml/min) around an extruding stream of the precursor cell suspension (flow rate 0.5 ml/min). The microdriplets gelled rapidly on being exposed to the laser light and were collected in a container containing PBS. The oil separated from the aqueous phase and the microspheres could be collected in the PBS below. The microspheres formed were thoroughly washed with PBS buffer to remove unreacted monomer and residual initiator. The size and shape of microspheres was dependent on extrusion rate and extruding capillary diameter (18 Ga to 25 Ga). The polymerization times were dependent on initiator concentration (ethyl eosin 5 μM to 0.5 mM, vinyl pyrrolidone (0.001% to 0.1%), and triethanolamine (5 mM to 0.1 M), laser power (120 mW to 2W), and monomer concentration (>10%w/v). Spheres prepared using this method had a diameter from 500 μm to 1,200 μm. The polymerizations were carried out at physiological pH in the presence of air. This is significant since radical polymerizations may be affected by the presence of oxygen. Cell viability subsequent to

encapsulation was checked by trypan blue exclusion assay and the encapsulated cells were found to be more than 95% viable after encapsulation.

Example 19: Various Formulations for the Prevention of Post Operative Adhesions.

The utility of PEG-oligo(α-hydroxy acid) diacrylates and tetraacrylates to prevent postoperative adhesions was evaluated in the rabbit uterine horn model as described above. The following polymers were synthesized, as described above: PEG 6K lactide diacrylate (6KL), PEG 10K lactide diacrylate (10KL). PEG 18.5K lactide (18.5KL), PEG 20K lactide (20KL). Solutions with 24% polymer in PBS with 900 ppm 2,2-dimethoxy-2-phenyl acetophenone, were prepared as described above. The solutions were applied to the uterine horn after cautery of the vascular arcade and illuminated with a 365 nm LWUV lamp, as described above. In one formulation, 18.5KL, 5 mg t-PA was mixed into the solution before application. Controls consisted of animals manipulated and cauterized but not treated with macromer solution. Measurement was performed on the 14th ± 1 day. Extent of adhesion was estimated from the fraction of the horn that was involved in adhesions, and the tenacity of adhesions was scored as 0, no adhesions; 1, filmy adhesions that offer no resistance to dissection; 2, fibrous adhesions that are dissectable by hand; 3, fibrous adhesions that are dissectable by blunt instruments; and 4, fibrous adhesions that are dissectable by sharp instruments. The results were as follows, where the extent of adhesions and the tenacity of the adhesions are shown.

Table 12: Efficacy of Polymer in Preventing Adhesions.

Formulation	Number of animals	Extent, %, ± S.D.	Tenacity, 0-4 ± S.D.
6KL	7	0.9 ± 1.7	0.9 ± 0.7
10KL	7	0 ± 0	0 ± 0
20KL	6	4.4 ± 5.0	0.9 ± 0.7
18.5KL t-PA	7	8.9 ± 13.1	1.6 ± 1.3
Control	7	35 ± 22	3.3 ± 0.6

Example 20: Polymerization of Ultrathin layers of Polymer on the surface of blood vessels to reduce thrombosis after vessel injury.

Blood vessels were harvested from rats and were rinsed free of blood. The endothelium of the vessel were removed by inserting a wooden dowel and rotating the vessel over the dowel. One vessel was used as a control, and was exposed to flowing blood as described below without further modification. Another vessel was treated first by exposure to eosin Y at 1 mM in saline, then rinsed in HEPES buffered saline, then filled with a solution of PEG-MA, PEG 10K with acrylate end-capped oligomers of DL lactide, containing triethanolamine (TEA) (100 mM) and N-vinylpyrrolidone (VP) (0.15%) and then illuminated by exposure to an argon ion laser at 0.5 W/cm2 for 15 sec. The nonpolymerized prepolymer mixture in the lumen of the vessel was rinsed away with saline. Human blood was collected from the antecubital vein and was anticoagulated with heparin at 2 units/ml. This blood was perfused through each vessel by a syringe pump at a flow rate corresponding to a wall shear rate of approximately 200/s for 7 min. The vessel was then superficially rinsed in saline and fixed in formaldehyde.

The treated vessel did not appear colored or different in color after perfusion compared to its color before perfusion, while the untreated control vessel appeared blood red. Thin segments of each vessel were cut from each vessel, were mounted on end, and were examined by environmental scanning electron microscopy (ESEM). ESEM is performed on hydrated samples in relatively low vacuum. This permits the visualization of the polymer film coating in the swollen and wet state. This is important to obtain measurements that may be readily interpreted, since the polymer film is approximately 95% water. A high degree of thrombosis was readily observed in the control vessel. The lumen of this vessel was narrowed to less than one-third its diameter pre-perfusion by the accumulation of thrombus, as shown in Figure 6A. By contrast, no thrombus could be observed in the lumen of the treated vessel, as shown in Figure 6B. A higher magnification of the vessel wall demonstrated no adherent thrombus. A still higher magnification shows a white structure which is the polymer film, which is different in contrast from the tissue due to differential charging under the electron beam of the ESEM. The film may be seen to be precisely conformed to the shape of the vessel and be approximately 5 - 8 μm thick.

The region of polymerization was restricted to the neighborhood of the blood vessel wall surface. The photosensitive dye was adsorbed to the vessel wall. Unbound dye was rinsed away. The entire lumen was filled with prepolymer, but upon illumination the gel formation was restricted to the vessel wall where the dye and the prepolymer meet. This interfacial polymerization process can be conducted to produce surface adherent layers that vary in thickness from less than 7 μm to more than 500 μm.

The above procedure was performed in 8 control rat arteries, and 8 treated arteries, with equivalent light microscopic histological results as described above. As demonstrated by this study, PEG prepolymers can be polymerized upon the lumenal surface of blood vessels. The immediate effect of this modification is to reduce the thrombogenicity of an injured blood vessel surface. This has clear utility in improving the outcome of balloon angioplasty by reducing the thrombogenicity of the vessel and lesion injured by balloon dilation. Another effect of this modification is to be reduce smooth muscle cell hyperplasia. This may be expected for two reasons. First, platelets contain a potent growth factor, platelet-derived growth factor (PDGF), thought to be involved in post-angioplasty hyperplasia. The interruption of the delivery of PDGF itself poses a pharmacological intervention, in that a "drug" that would have been delivered by the platelets would be prevented from being delivered. Thrombosis results in the generation of thrombin, which is a known smooth muscle cell mitogen. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. There are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The interruption of thrombin generation and delivery to the vessel wall also poses a pharmacological intervention. Moreover, there are other growth factors soluble in plasma which are known to be smooth muscle cell mitogens. The gel layer is known to present a permselective barrier on the surface of the tissue, and thus the gel layer may reasonably be expected to reduce hyperplasia after angioplasty. The inhibition of thrombosis upon the vessel wall may also reduce the incidence of abrupt reclosure and vasospasm, both of which occur sometimes following vascular intervention.

Example 21: Interfacial Polymerization of Macromers Inside Blood Vessels to Prevent Thrombosis.

Macromer solutions were polymerized interfacially within previously injured blood vessels *in vivo* to prevent thrombosis. The carotid artery was exposed, and a polyethylene tube (PE-10) was used to cannulate the exterior carotid artery. The artery was clamped with fine arterial clamps proximal to the interior/exterior carotid artery bifurcation and approximately 2 cm distal to the bifurcation. A 1 ml tuberculin syringe was used to rinse the blood from the lumen of the isolated zone by filling and emptying the vessel zone. The vessel was injured by crushing using a hemostat. The isolated zone was filled with a 10 mM solution of eosin Y for 2 minutes, after which it was rinsed and filled with a 20% solution of a macromer in saline with 0.1 mM triethanolamine and 0.15% N-vinyl pyrrolidinone. The macromer consisted of a PEG chain of MW 8,000 daltons, extended on both sides with a lactic acid oligomer of an average degree of polymerization of 5 lactidyl groups, and further acrylated nominally at both ends by reaction with acryloyl chloride. The vessel was illuminated transmurally using an argon ion laser (514 nm) at an intensity of approximately 1 mW/cm^2 for 5 seconds. Following this, the cannula was removed from the exterior carotid artery and the artery was ligated at the bifurcation. The arterial clamps were removed to permit the resumption of blood flow. Perfusion was allowed for 20 minutes, following which the vessel were again isolated, removed from the body, gently rinsed, fixed, and prepared for light microscopic histological analysis. Using the naked eye, the crushed segments in control animals, which lacked illumination, were red, indicating internal thrombus with entrapped red blood cells. By contrast, no

redness was observed at the site of the crush injury in the treated vessels. Histology showed extensive thrombus, fibrin, and entrapped red blood cells in the non-treated vessels. By contrast, no thrombus or fibrin or entrapped red blood cells were observed in the treated vessels. The procedure was conducted in four control animals and three treated animals.

This example demonstrates that the polymerization can be carried out *in situ* in the living animal, that the polymer coating remains adherent to the vessel wall during arterial blood flow, and that the polymer coating can prevent thrombosis *in vivo* in non-anticoagulated animals. This approach to treatment has clear benefits in preventing abrupt reclosure, vasospasm, and restenosis after intravascular interventional procedures. Moreover, it is more generally applicable to other intraluminal and open-surface organs to be treated.

Modifications and variations of the present invention, the macromer and polymeric compositions and methods of use thereof, will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the appended claims.

We claim:

1. A biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region.

2. The macromer of claim 1 wherein the water soluble region is attached to a degradable region, at least one polymerizable region is attached to the water soluble region, and at least one polymerizable region is attached to the degradable region.

3. The macromer of claim 1 wherein the water soluble region forms a central core, at least two degradable regions are attached to the core, and at least two polymerizable regions are attached to the degradable regions.

4. The macromer of claim 2 wherein the degradable region is a central core, at least two water soluble regions are attached to the core, and at least one polymerizable region is attached to each water soluble region.

5. The macromer of claim 1 wherein the water soluble region is a macromer backbone, the degradable region is a branch or graft attached to the macromer backbone, and at least two polymerizable regions are attached to the degradable regions.

6. The macromer of claim 1 wherein the degradable region is a macromer backbone, the water soluble region is a branch or graft attached to the degradable backbone, and two or more polymerizable regions are attached to the water soluble branches or grafts.

7. The macromer of claim 1 wherein the water soluble region is a star backbone, the degradable region is a branch or graft attached to the water

soluble star backbone, and at least two polymerizable regions are attached to a degradable branch or graft.

8. The macromer of claim 1 wherein the degradable region is a star backbone, the water soluble region is a branch or graft attached to the degradable star backbone, and two or more polymerizable regions are attached to the water soluble branch or graft.

9. The macromer of claim 1 wherein the water soluble region is also the degradable region and two or more polymerizable regions are attached to the water soluble regions.

10. The macromer of claim 1 wherein the water soluble region is also the degradable region, one or more additional degradable regions are grafts or branches upon the water soluble region, and a total of two or more polymerizable regions are attached to the degradable regions.

11. The macromer of claim 1 comprising a core, at least two extensions on the core, and an end cap on at least two extensions, wherein

the core comprises poly(ethylene glycol).

12. The macromer of claim 11 wherein

each extension comprises biodegradable poly(hydroxy acid); and

each end cap comprises an acrylate oligomer or monomer.

13. The macromer of claim 12 wherein the poly(ethylene glycol) has a molecular weight between about 400 and 30,000 Da;

the poly(hydroxy acid) oligomers have a molecular weight between about 200 and 1200 Da; and

the acrylate oligomer or monomer have a molecular weight between about 50 and 200 Da.

14. The macromer of claim 1 wherein the polymerizable regions contain a carbon-carbon double

bond capable of cross-linking and polymerizing macromers.

15. The macromer of claim 1 wherein crosslinking and polymerization of the macromer are initiated by a light-sensitive free-radical polymerization initiator with or without a cocatalyst, further comprising a free radical polymerization initiator.

16. The macromer of claim 15 wherein the initiator is selected from the group consisting of xanthine dyes, acridine dyes, thiazine dyes, phenazine dyes, camphorquinone dyes, and acetophenone dyes.

17. The macromer of claim 16 wherein the initiator is selected from the group consisting of an eosin dye with triethanolamine, 2,2-dimethyl-2-phenyl acetophenone, and 2-methoxy-2-phenyl acetophenone.

18. The macromer of claim 1 wherein crosslinking or polymerizations are initiated *in situ* by light having a wavelength of 320 nm or longer.

19. The macromer of claim 1 wherein the degradable region is selected from the group consisting of poly (hydroxy acids), poly(lactones), poly(amino acids), poly(anhydrides), poly(orthoesters), poly(phosphazines), and poly(phosphoesters).

20. The macromer of claim 19 wherein the degradable region is a poly(α-hydroxy acid) selected from the group consisting of poly(glycolic acid), poly(DL-lactic acid) and poly(L-lactic acid).

21. The macromer of claim 19 wherein the poly(lactone) is selected from the group consisting of poly(ε-caprolactone), poly (δ-valerolactone) or poly(λ-butyrolactone).

22. The macromer of claim 1 wherein the water soluble region is selected from the group consisting of poly(ethylene glycol), poly(ethylene oxide), poly(vinyl alcohol), poly(vinylpyrrolidone),

poly(ethyloxazoline), poly(ethylene oxide)-co-poly(propyleneoxide) block copolymers, polysaccharides, carbohydrates, proteins, and combinations thereof.

23. The macromer of claim 1 further comprising biologically active molecules selected from the group consisting of proteins, carbohydrates, nucleic acids, organic molecules, inorganic biologically active molecules, cells, tissues, and tissue aggregates.

24. A method of forming a polymeric, biocompatible material on tissue comprising

applying to the tissue a solution of biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region, in the presence of a free radical initiator, and polymerizing the macromer.

25. The method of claim 24 wherein the tissue is coated to prevent adhesion of the tissue to other tissue.

26. The method of claim 24 wherein the tissue is coated and adhered to other tissue during polymerization.

27. The method of claim 24 further comprising providing with the macromer solution biologically active molecules selected from the group consisting of proteins, carbohydrates, nucleic acids, organic molecules, inorganic biologically active molecules, cells, tissues, and tissue aggregates.

28. The method of claim 24 further comprising first applying a free radical initiator at the site where the macromer solution is to be polymerized.

29. The method of claim 28 wherein the initiator binds to the tissue, further comprising

removing unbound initiator prior to application of the macromer solution.

30. A method for controlled release of biologically active molecules comprising mixing biologically active molecules with a solution of biodegradable, polymerizable, and at least substantially water soluble macromer comprising at least one water soluble region, at least one degradable region, and at least two free radical polymerizable regions, wherein the polymerizable regions are separated from each other by at least one degradable region, in the presence of a free radical initiator, and polymerizing the macromer to entrap the molecules within the resulting polymer.

31. The method of claim 30 wherein the polymer forms a shape selected from the group consisting of microspheres, sheets, rods, and particles.

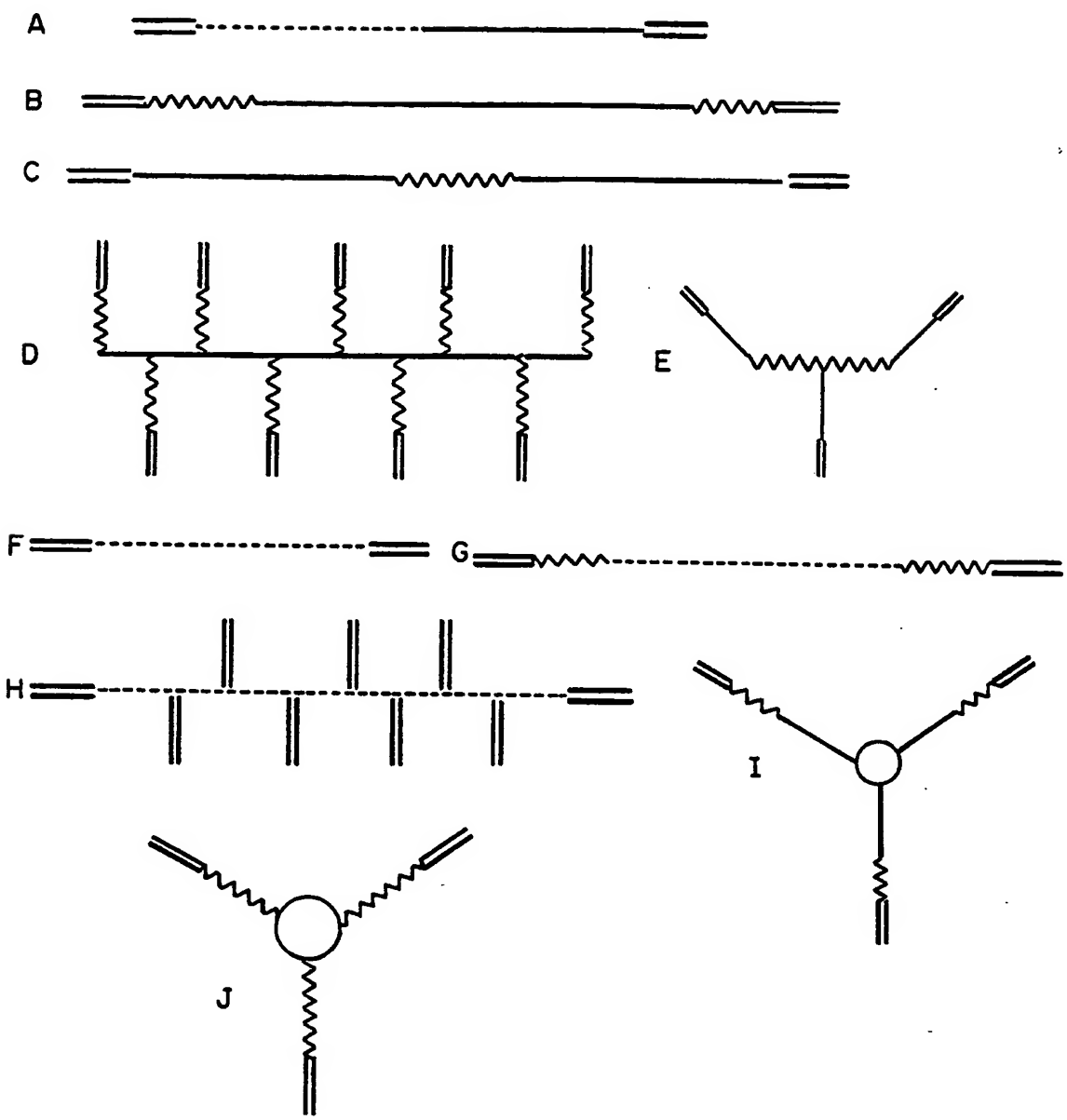
A
B
C
D
E
F
G
H
I
J
- WATER SOLUBLE COMPONENT
- HYDROLIZABLE COMPONENT
- WATER SOLUBLE AND HYDROLIZABLE COMPONENT
- PHOTOPOLYMERIZABLE COMPONENT

FIG. 1

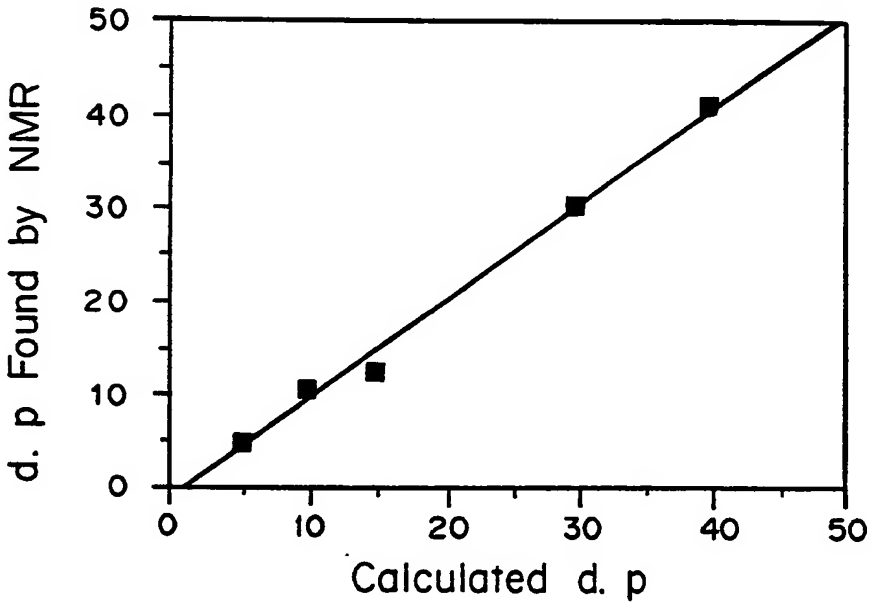

FIG. 1a

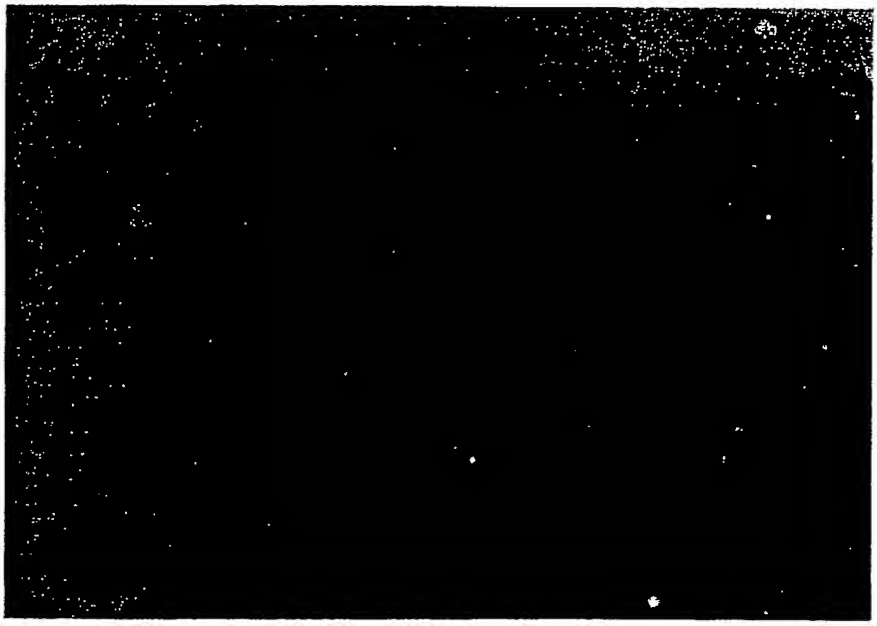

FIG. 2a

FIG. 2b

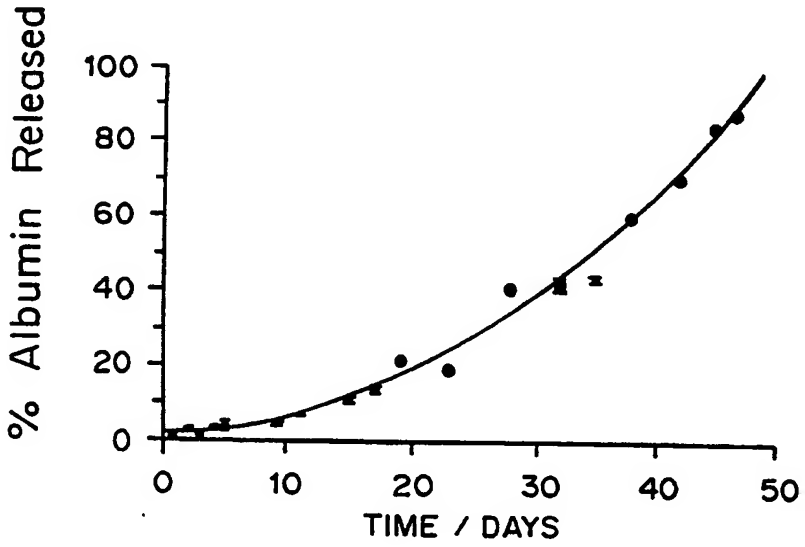
% Albumin Released
100
80
60
40
20
0
0
10
20
30
40
50
TIME / DAYS

FIG. 3a

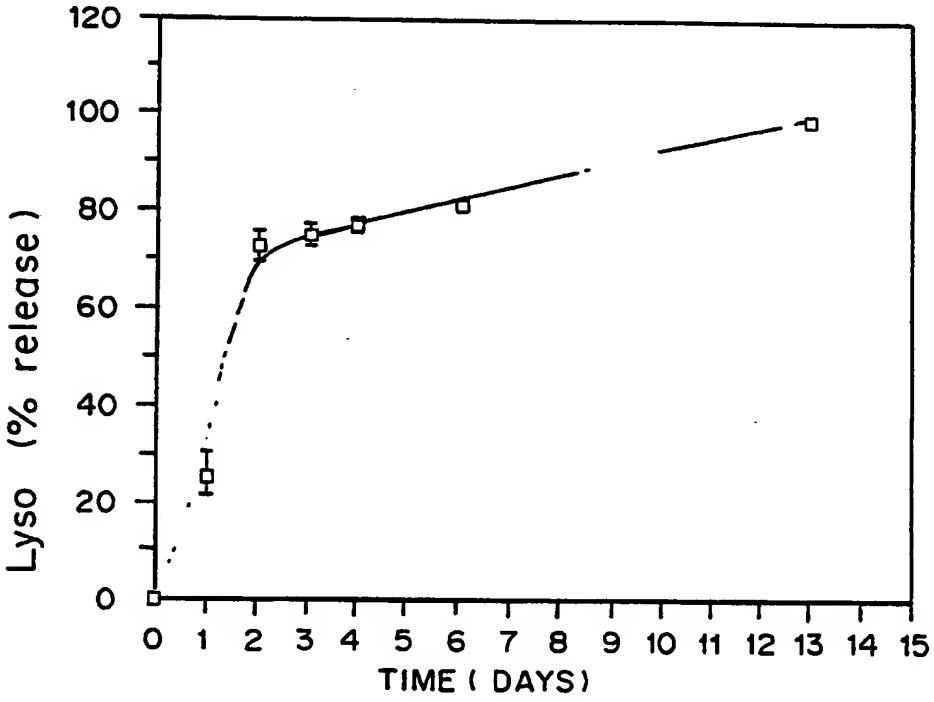
Lyso (% release)
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (DAYS)

FIG. 3b

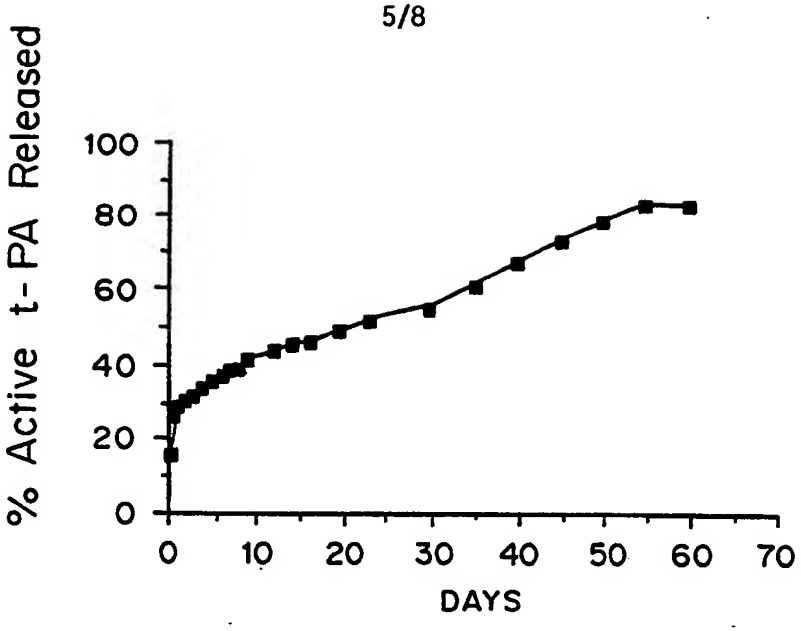
% Active t-PA Released
100
80
60
40
20
0
0
10
20
30
40
50
60
70
DAYS

FIG. 4a

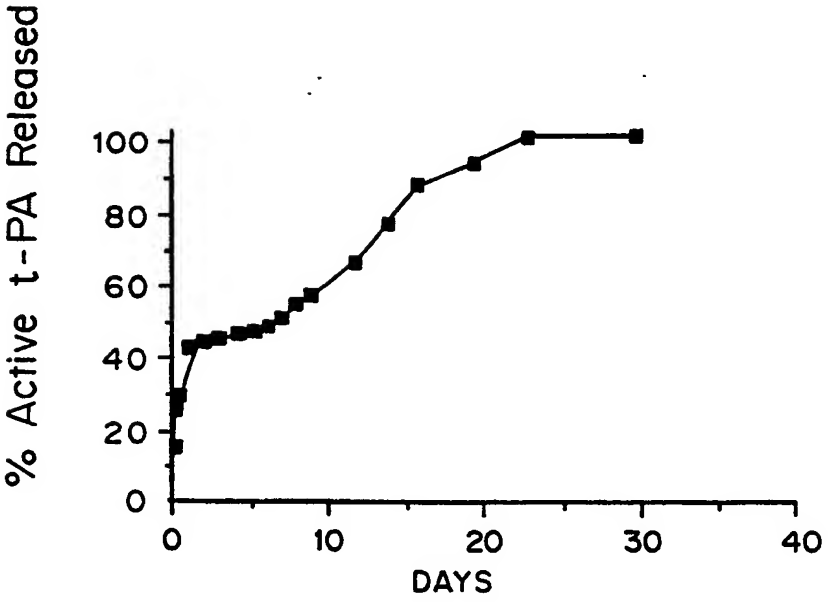
% Active t-PA Released
100
80
60
40
20
0
0
10
20
30
40
DAYS

FIG. 4b

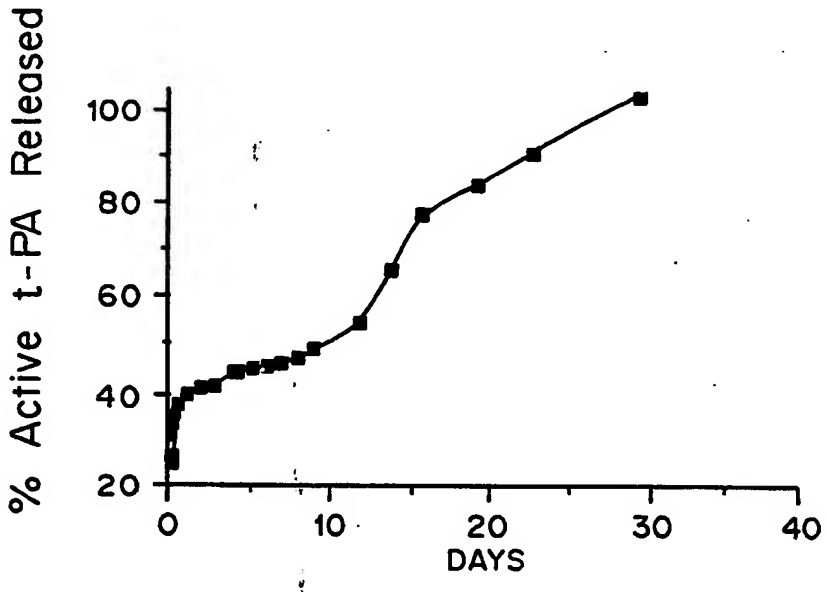

FIG. 4c

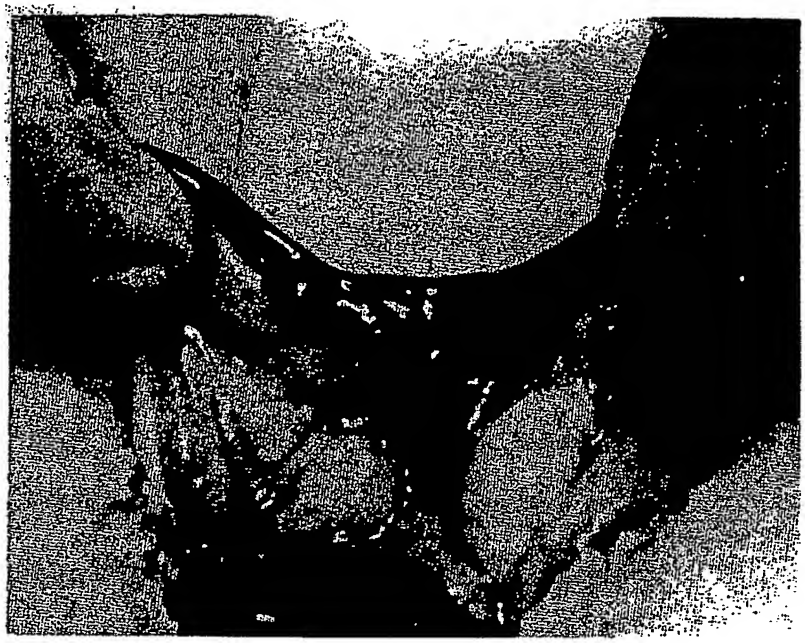

FIG. 5a

FIG. 5b

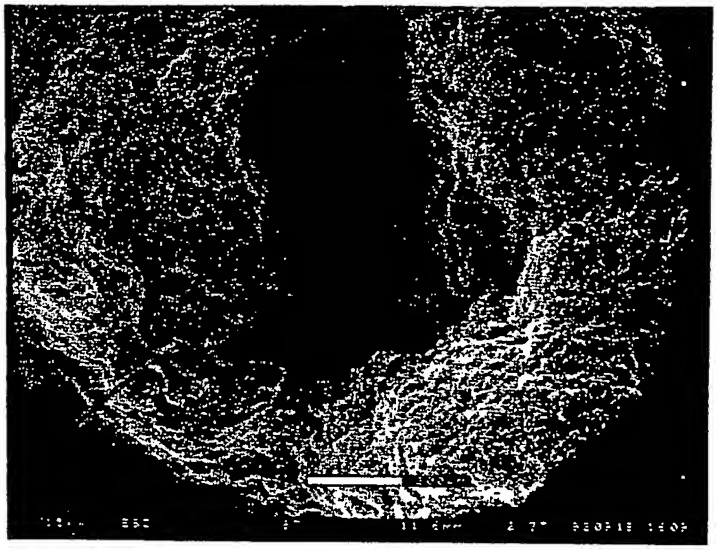

FIG. 6a

FIG. 6b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01773

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 9/50
US CL :522/26, 43, 56, 68, 87, 88, 149, 171, 181; 424/423, 426, 489
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 522/26, 43, 56, 68, 87, 88, 149, 171, 181; 424/423, 426, 489

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,310,397 (KAETSU ET AL) 12 JANUARY 1982. See entire document.	1-31
A	US, A, 4,994,277 (HIGHAM ET AL.) 19 FEBRUARY 1991. See entire document.	24-31
A,P	US, A, 5,135,751 (HENRY ET AL.) 04 AUGUST 1992. See entire document.	24-31
A,P	US, A, 5,108,755 (DANIELS ET AL.) 28 APRIL 1992. See entire document.	24-31
A	US, A, 4,999,417 (DOMB) 12 MARCH 1991 See entire document.	24-31

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 APRIL 1993	02 AUG 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer AMY HULINA
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-2351

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/01773

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,512,910 (SCHMIDLE) 23 APRIL 1985 See entire document.	1-23
A	US, A, 4,533,445 (ORIO) 06 AUGUST 1985 See entire document.	1-23